PHYSIOLOGY AND ENERGETICS OF THE SANDY-BEACH BIVALVE *DONAX SERRA* RÖDING WITH SPECIAL REFERENCE TO TEMPERATURE AND CHLORINE TOLERANCE

by

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DECLARATION

I hereby declare that the conception, execution and final synthesis of the work embodied in this thesis was undertaken solely by myself. No co-authors were involved in the writing of any part of this thesis. Certain persons acted as invaluable referees and they are thanked individually under the acknowledgements. If data collected by any other person were used in any part or form to help in the interpretation of my own work, this was duly declared in the relevant section.

Jeanie Stenton-Dozey

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ABSTRACT

This thesis examines the physiology and energetics of *D. serra* and considers physiological responses to elevated temperature and chlorine levels typical of sea water discharged from a nuclear power station. The first chapter presents a brief review of studies relating to the biology of *D. serra* and identifies a conspicuous lack of information on the physiology of this species. This chapter also describes thermal stratification and dispersion of the discharged water in the vicinity of the sandy beach inhabited by *D. serra*. This allows results relating to temperature and chlorine tolerance to be correlated with the microgeographical distribution of *D. serra*.

SECTION 1 of this thesis concentrates on basic physiological characteristics such as survival rates, burrowing behaviour, heart rate (Chapter 2) and biochemical composition (Chapter 3).

In Chapter 2, median lethal time and median lethal temperature were used to define the size-related upper thermal tolerance of *D. serra*. Results demonstrated that small-sized individuals would best tolerate heated effluent from the power station. Temperatures above 32°C were lethal to all sizes of *D. serra*. After extended exposure to temperatures between 24°C and 29°C, 50% of the animals no

longer remained buried. Since these temperatures occur in the thermal plume, such displacement from the sand can result in stranding on the beach.

According to median lethal times, *D. serra* tolerated chlorine levels < 0.3 ppm. The threshold between chronic and acute chlorine toxicity was 0.6 ppm. Above this concentration *D. serra* closed its valves for up to 6 - 8 days. Recovery hereafter was only marginal.

Heart beat frequency increased with thermal stress but was depressed in the presence of chlorine. Heart rates revealed sub-lethal effects at chlorine levels >0.6 ppm in combination with temperature >20°C. These results indicated that the plume conditions would not be lethal to *D. serra*, but sub-lethal effects relating to burrowing behaviour and heart rates can be expected.

In Chapter 3, changes in the biochemical composition of *D. serra* over a period of 90 days was investigated in relation to sub-lethal temperatures (15 to 25°C) and chlorine levels (0.1 - 0.3 ppm). Laboratory confinement under simulated natural conditions did not change the biochemical composition of *D serra*. Protein was identified as the main energy store, followed by carbohydrates.

High temperatures in the presence or absence of chlorine caused a rapid depletion of reserves. Generally, protein was utilised more readily and to a greater extent than carbohydrates. Continual loss of reserves throughout the 90-day experiment indicated non-acclimation to the

imposed conditions. Progressive degeneration of the gonads was closely coupled with the depletion of body and reproductive reserves. Conditions at the power station outfall are unlikely, however, to cause long-term changes in biochemical composition since exposure to temperature and chlorine levels as used in the present study would be intermittent and of short duration.

SECTION II of this thesis is concerned with physiological energetics which encompasses integration of rates of energy acquisition (Chapter 4) and expenditure (Chapter 5) to arrive at estimates of scope for growth and reproduction (Chapter 6). Chapter 7 considers the effects of temperature and chlorine on the physiological energetics of *D. serra*.

In chapter 4, the interactive effect of body size and diet on clearance, ingestion and absorption rates are considered. The availability of natural particulates to *D. serra* was found to be dependent on episodic upwelling and downwelling. The potential food resource comprised phytoplankton-derived detritus augmented by phytoplankton which bloomed in response to upwelling. In feeding experiments, cultured algae represented the high quality food value of phytoplankton whilst seafoam material was used to simulate the poorer quality of detritus.

Feeding rates were markedly influenced by food quality and quantity. The maximum amount of algae ingested was three times that of detritus. Irrespective of diet however,

ingestion was regulated by increasing clearance rates over an optimal food range concomitant with maxima in absorption efficiencies. On a heterogeneous detrital diet, pre-ingestive selection was not an apparent means of regulating the quality of the ingested ration, making it more likely that post-ingestive sorting determined the ultimate assimilation of detritus.

At high particulate densities regulation of ingestion and absorption rates depended on food quality. Detritus-fed animals reduced clearance rates together with a small release of pseudofaeces. Those fed algae reduced clearance and increased pseudofaeces production or increased clearance at the same time as egesting copious amounts of pseudofaeces and faeces containing an abundance of undigested material.

It is concluded that *D. serra* is an opportunistic filter-feeding in its natural environment. It can maximise particle ingestion at times of both a moderate and abundant supply of high quality food such as phytoplankton.

Chapter 5 considers variations in rates of respiration and ammonia excretion in relation to body size, diet and activity levels. Food quality had a significant effect on the relationship between body size and respiration rate as well as on absolute rates. With detritus as the food source, O_2 uptake rates while feeding were markedly reduced. Irrespective of diet, standard metabolism, rather than feeding activity, utilised the most energy. With respect to

feeding, post-ingestive processes were more costly than those associated with filtration.

The relationship between size and excretion rate was not influenced by activity levels or diet. Absolute rates were highest and O:N ratios lowest in starved individuals. This indicated considerable protein catabolism during nutritional stress and supported the conclusion reached from data on gross biochemical composition that *D. serra* relies predominantly on protein as an energy reserve.

Data from Chapters 4 and 5 were used in Chapter 6 to calculate the balance between energy gain and loss so that the scope for growth and reproduction (SFG) could be estimated in relation to body size and diet. Laboratory-based SFG was compared to SFG estimates determined from field measurements of shell width, tissue weight and growth cohorts covering a period of 5 years.

At comparable ration levels, SFG on an algal diet surpassed that when feeding on seafoam detritus. The principle reasons for this contrast were reduced ingestion rates, and to a lesser extent, lower absorption efficiencies in detritus-fed animals. This suggested significant short-term optimisation of SFG and growth efficiencies in the presence of high quality food material.

Cohort analysis of field data produced a Gompertz growth curve which demonstrated that asymptotic body size is reached after 5-6 years and that growth rate declines with age. Field data also established the partitioning of total

production between somatic and reproductive growth. All these features were not apparent in laboratory-based estimates of SFG.

Optimal SFG, growth efficiencies and P:B ratios on an algal diet grossly overestimated natural production, whereas with detritus as a food source, moderate underestimations resulted. The validity of using laboratory-based SFG as an index of real growth was concluded to be a function of the combination of food quality and quantity.

Chapter 7 deals with the effects of temperature and chlorine on clearance and ingestion rates, absorption efficiencies, rates of respiration and excretion and estimates of SFG when *D. serra* was feeding at the optimum algal ration. Feeding rates were independent of acclimation temperature but absorption efficiencies declined sharply with temperature elevation. Chlorination resulted in a decrease in clearance and ingestion rates, but at the same time these reduced rates became temperature-dependent. The temperature-related decline in absorption efficiencies remained unchanged in the presence of chlorine.

The magnitude of increase in metabolic expenditure in relation to raised temperature was such that partial acclimation was indicated. The addition of chlorine resulted in reduced, but significantly temperature-dependent respiration rates. Excretion rates also slowed down in the presence of chlorine.

SFG and growth efficiencies, although always positive, declined with an increase in temperature. A combination of high temperature and chlorine resulted in marginally negative or positive SFG. This suggested that within the influence of the discharge plume, the growth rate and the maximum attainable body size of *D. serra* may decline. As a consequence of a reduction in body size, reproductive output would be less, although there is indirect evidence that reproductive effort per individual would be maintained.

Finally, in Chapter 8, the results of this thesis are synthesised and information relevant to improving the knowledge of marine bivalve physiology is summarised. Final conclusions are reached on the extent to which the discharge plume from the power station may influence the physiology of *D. serra*.

CHAPTER ONE

INTRODUCTION

The burrowing bivalve *Donax serra* Röding is an important and often dominant component of sandy beaches along the southern African coastline. Its geographical range extends from Namibia on the west coast, round the Cape, to the Transkei in the south-east (Day, 1974) - a span of some 2000 km. *D. serra* is most often found on clean, high-energy beaches in the intertidal zone, with the centre of the population being located near the low tidal limit. It is the largest member of its genus, attaining a shell length of 80 mm and a dry mass of 5 to 6 g for a single individual. Very large populations occur along the west coast and in the Algoa Bay region. In these areas *D. serra* comprises 80 - 98% of the total macrofaunal biomass. Maximum densities of 1700 m^{-2} and biomass values of up to 986 g dry tissue weight m^{-2} have been encountered (McLachlan, 1977a, b; Bally, 1981; Cook & Birkett, 1984).

The ecology of sandy beaches in South Africa has been the focus of extensive research, especially along the southern Cape west coast and around Algoa Bay. *Inter alia* considerable knowledge has been gained on the ecology of *D. serra* such as shoreline distribution, population growth and production, reproductive cycles and migration patterns (de Villiers, 1975a, b; McLachlan 1977a, b, 1980; McLachlan & Hanekom, 1979; McLachlan *et al.*,1979; Bally, 1981; Hutchings *et al.*, 1983; Cook & Birkett, 1984, 1986; Donn, 1986; Donn *et al.*, 1986; Birkett & Cook, 1987; Donn, 1987). Arising

from these studies have been assessments of the contribution of *D. serra* to nutrient regeneration in the surf zone (Prosch & McLachlan, 1984) as well as to carbon (McLachlan & Bate, 1984) and nitrogen (Cockcroft, 1988) budgets.

By contrast to the vast amount of information on the ecology of *D. serra*, little is known about its general biology and even less about its physiology. Survival rates and burrowing responses have been investigated at high (Ansell & McLachlan, 1980) and low (McLachlan & Young, 1982) temperatures. Other aspects of locomotory activity considered have been the dynamics of pedal extension (Trueman & Brown, 1985) and blood flow (Trueman *et al.*, 1986) and respiration (Trueman & Brown, 1987) have been measured in the foot while burrowing. Healing and regeneration of siphons (Hodgson, 1982) and the structure and distribution of ciliated receptors (Hodgson & Fielden, 1984) have also received attention.

The functional morphology of feeding was examined by Ansell (1981) and the role of digestive enzymes and gut bacteria in food assimilation was evaluated by Krohn (1987). The most recent research on feeding has been on clearance and yield of bacterioplankton and suspended particulates for *D. serra* (Matthews *et al.*, 1989). Two rather elementary studies have been undertaken on aerobic respiration rates, one in relation to temperature (Dye, 1979) and the other to oxygen tension (Van Wijk *et al.*, 1989).

The above studies present a fragmented overview of the biology of *D. serra* which is notably deficient in information on the physiological energetics of the species. An ideal opportunity to remedy this presented itself with the construction of the first South African nuclear power station in the immediate vicinity of an expansive sandy beach densely populated by *D. serra*. A study of the impact of power plant discharges on the physiological fitness of *D. serra* was invited by the National Marine Pollution Monitoring Programme.

The Koeberg Nuclear Power station is situated on the Cape southwest coast approximately 35 km north of Cape Town (Fig. 1.1). The coastline in this area is completely exposed and subjected to vigorous wave action. The beach has an extensive surf zone of up to 1 km due to the gentle slope of the sea-bed of about 1:100 (Rattey & Potgieter, 1987). Ambient temperatures in the surf zone are strongly dependent upon the dynamics of upwelling. Coldest temperatures (8 - 10°C) follow upwelling which is induced by strong southerly or southeasterly winds mostly in the summer months. In winter there are more northwesterly winds, which force warmed offshore surface waters of 15 - 17°C to advect onshore resulting in downwelling.

The power station uses sea water as a cooling medium to condense exhaust steam from the turbines. Sea water is pumped from an intake basin and discharged directly into the surf zone via an outfall channel 200 m to the south (Fig.

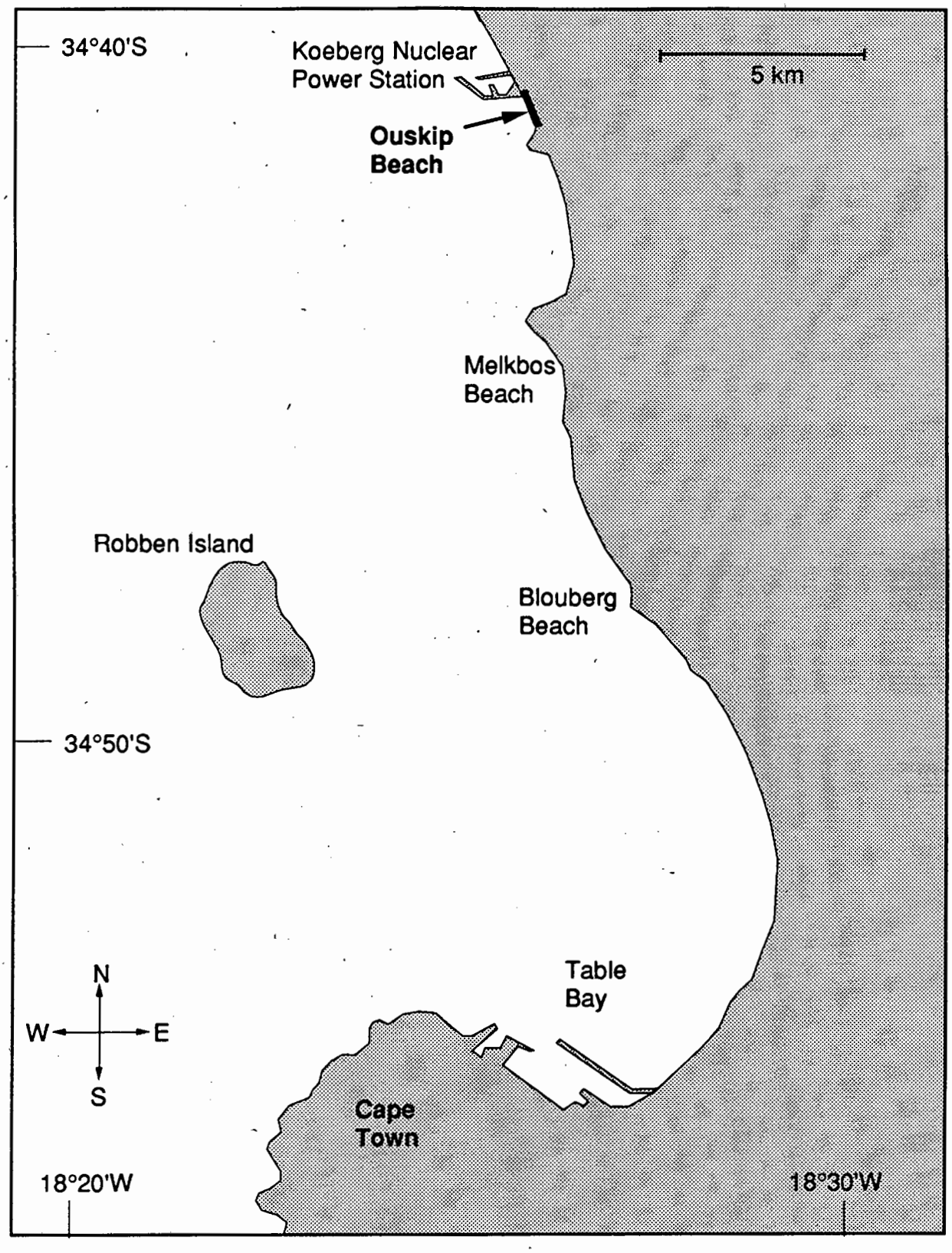

Fig 1.1. The locality of Ouskip Beach relative to the position of Koeberg Nuclear Power Station and Cape Town. All experimental animals were collected from the southern end of Ouskip beach.

1.2). When the power station is running at full load the flow rate of the cooling water is 80 m^3 sec^{-1} and 10°C warmer than ambient sea water temperature. A warm water plume, with thermal gradients ranging from ambient temperatures to 23 - 25°C, is thus created.

Dissipation of warm water is primarily reliant on turbulent mixing by breaking waves in the surf zone, but is also dependent on wind direction (Fig. 1.2). During an onshore NW wind, the plume movement is restricted to the coastline for some time and is subject to the waves and currents in the surf zone. By contrast a SE wind forces warmed water to advect offshore where the plume is rapidly dispersed by diffusion and conduction. The spatial extent of possible thermal stress to *D. serra* from the plume is thus dependent on an interaction between inlet (ambient) temperature, the degree to which sea water is heated and wind direction (with the associated wave activity and nearshore currents).

The power station discharge also introduces a variable load of chlorine into the surf zone. Sea water has a high chlorine demand involving immediate reactions with organic matter, dissolved gases and inorganic salts with the result that residuals and halogenated derivatives are rapidly formed in sea water (Morgan & Carpenter, 1978). Free residual concentrations measured at the outfall do not normally exceed 0.5 ppm, but can reach 2.0 ppm if shock dosing is undertaken. Once the discharged water reaches the

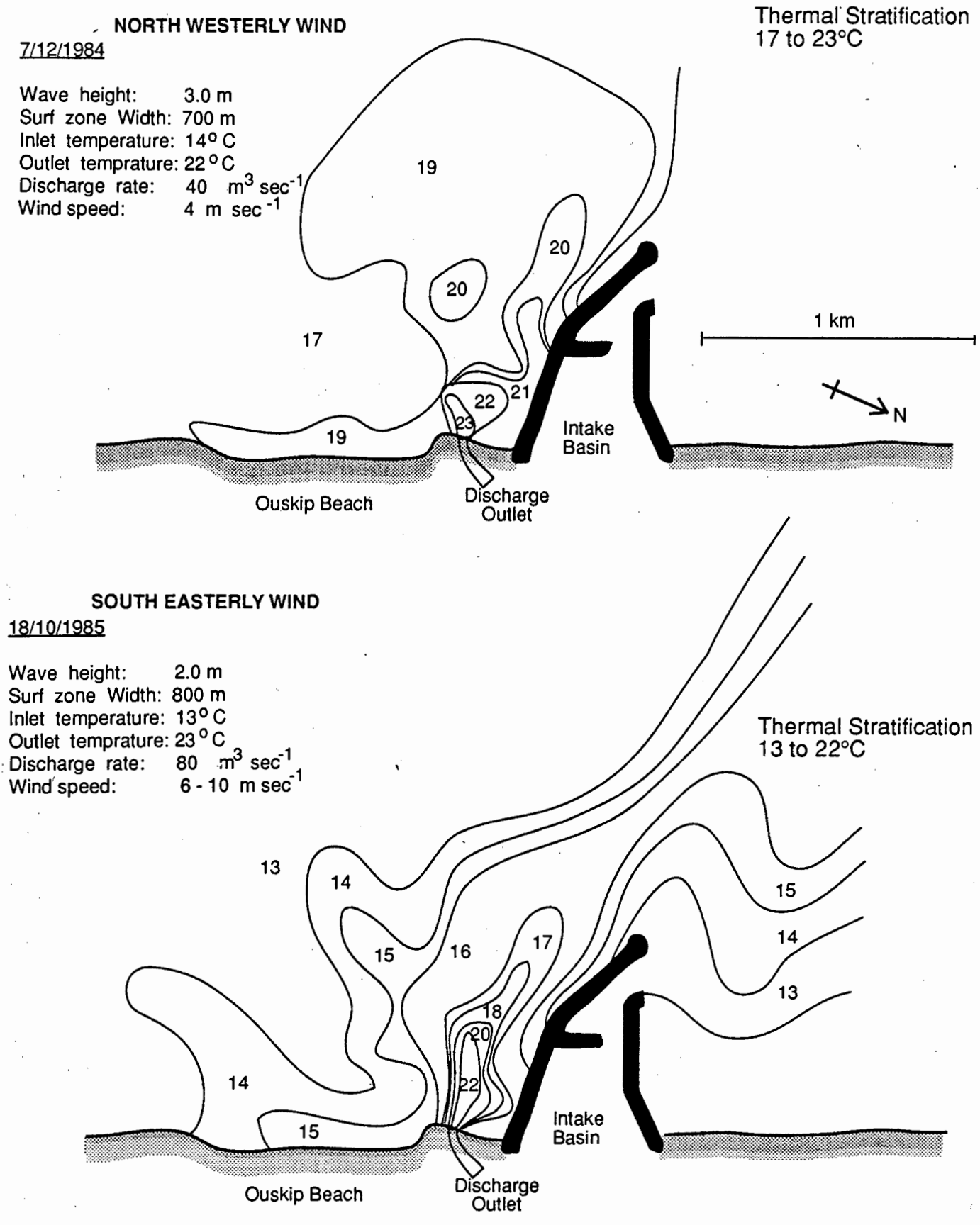

Fig 1.2. Thermal stratification of plumes from discharge outlet of Koeberg Nuclear Power Station during a north-westerly & a south-easterly wind. (after Rattey & Potgieter, 1987).

surf zone however, chlorine residuals are rapidly diluted (Rattey & Potgieter, 1987) so that spatially, the possible impact of these residuals on *D. serra* would be more limited than that of the thermal plume. The fate of the halogenated residuals is not known.

Before the effects of the chlorinated warm water discharge on *D. serra* can be considered, natural physiological traits, including intrinsic variations in rate functions require investigation. There are thus two distinct aspects to the research presented in this thesis. Fundamental studies were undertaken into basic physiological characteristics, such as burrowing behaviour, valve movements and heart rate. This was followed by detailed research into physiological energetics which encompassed the integration of rates of energy acquisition and expenditure to arrive at estimates of scope for growth and reproduction. The second aspect considers the effects of temperature and chlorine on these physiological attributes of *D. serra* at levels characteristic of the discharged sea water. The thesis is thus divided into two sections, the first focusing on basic physiology and the second on physiological energetics. Each section incorporates relevant research on the effects of temperature and chlorine.

S E C T I O N I

BASIC PHYSIOLOGY

CHAPTER TWO

EFFECTS OF TEMPERATURE AND CHLORINE ON SURVIVAL, BURROWING RESPONSE AND HEART RATE

INTRODUCTION

The extensive research on thermal tolerances of marine organisms has focused strongly on the survival of fish larvae and juveniles near power plants using sea water as a coolant (reviewed by Schubel *et al.*, 1978) and from a different perspective, the ways in which intertidal animals survive desiccation and increases in temperature during aerial exposure (reviewed by Newell, 1979). This research has shown that the thermal tolerance of an organism is a product of an interaction between exposure temperature and the duration of that exposure and it is imperative to take cognizance of both these factors in any experiments designed to determine upper lethal limits. Unfortunately, earlier data were obtained by slowly heating the water in which animals were contained at an arbitrary rate of 1°C in 5 to 10 mins (Huntsman & Sparks, 1924; Henderson, 1929; Broekhuysen, 1940; Evans, 1948; Southward, 1958; McLachlan & Erasmus, 1974). This method resulted in the estimation of upper lethal limits very much higher than would ever be encountered by the organisms in their natural environment (Newell, 1979).

The importance of the interaction between duration of exposure and temperature was recognised by researchers working on fish (see Schubel *et al.*, 1978) and the methods they established are now widely used to measure temperature tolerances of marine organisms in terms of both natural and man-induced changes. In this approach the time taken to

reach 50% mortality is determined or, alternatively, the temperature at which 50% mortality occurs can be used as the criterion of upper temperature tolerances (Kennedy & Mihursky, 1971). In this chapter both median lethal time and median lethal temperature are used to define the tolerances of *D. serra* in response to a range of temperatures equal to and greater than expected in the effluent of the power station. As mentioned in the introduction to this thesis, the discharged water is approximately 10°C warmer than ambient which results in the thermal plume potentially ranging in temperature from 18°C to 27°C.

Thermal tolerance of the *Donax* population near Koeberg on the west coast is compared with that of a population on the south coast of South Africa where the local temperature is higher and the intertidal distribution shows a reverse pattern with respect to size. In order to demonstrate the complex interaction between upper temperature tolerances and latitudinal and shoreline distributions, South African *Donax* species are compared to those in Europe from littoral and sublittoral habitats. In addition, further comparisons between *Donax* species and other genera of burrowing bivalves are used to illustrate some common tolerances to high temperatures.

Experiments were also undertaken to determine the survival as median lethal time of *D. serra* exposed to chlorine, a defouling agent used in the condenser pipes and

present in the effluent in amounts varying from 0.1 ppm to 0.3 ppm, but with the potential of reaching 2.0 ppm during shock dosing. The ecological advantage of a rapid response and subsequent isolation of internal tissues by *D. serra* and other bivalves in the presence of chemical pollutants is discussed in terms of the role played by sensory receptors on the siphons and mantle edge.

A common physiological measure of the response of bivalves to raised temperatures, as well as to the presence of chemical pollutants, is the change in heart activity (Coleman, 1974 (review); Lowe, 1974; Earll, 1975; Trueman & Akberali, 1981; Trueman, 1983). Since heart rate is easy to monitor using impedance techniques (Trueman *et al.*, 1973), it has proved a popular measure of gross changes of behaviour, such as valve movements, burrowing and moments of complete inactivity or heightened activity. This behaviour in turn corresponds closely to changes in the physical and chemical environment (Earll, 1975). In this chapter heart rate is used as a possible indicator of stress in *D. serra* in response to temperature both in the presence and absence of chlorine.

Lethal and sub-lethal thresholds determined in this chapter are subsequently used in the second section of this thesis to set the sublethal temperature and chlorine ranges for research into their effects on the physiological energetics of *D. serra*.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE

Bivalves were collected from Ouskip beach south of the power station outfall but distant enough so that the influence of the thermal plume was unlikely to be significant. Animals were kept in flowing sea water in 25-l tanks which were fitted with air-lift pumps to facilitate circulation of water at 15°C through a bed of sand and gravel. As water in the aquarium was replaced regularly, natural detritus was available as food, but this was supplemented by periodic additions of cultured algae (*Tetraselmis suecica*). There was automatic control of a day:night photoperiod in the aquarium housing the tanks. Animals were used within two weeks of collection.

Six separate experiments were designed to investigate the effect of acute and stepwise changes in temperature on survival and valve adduction, the effect of different chlorine concentrations on survival rates and finally, the effect of temperature with and without chlorine on heart rate.

SURVIVAL EXPERIMENTS

Acute temperature exposure

In this experiment an attempt was made to simulate acute thermal shock similar to that which arises during short term temperature changes at the power station. These changes had to exceed natural swift variations in ambient temperatures to which *D. serra* may be acclimatised. For example, a rapid increase in temperature can be expected when the prevailing wind shifts to an onshore north-westerly which concentrates the thermal plume in the surf zone near the outfall (Rattey & Potgieter, 1987). Sudden changes in temperature can also result from emergency shutdowns of the nuclear power station as occurred in early 1985.

The temperature at which 50% of test animals died after acute exposure to a particular thermal level for 4 days, was defined as the median lethal temperature (LT_{50}). This term was used by Kennedy & Mihursky (1971) in their work on estuarine bivalves. Median lethal time, that is the time to 50% mortality at a particular exposure temperature (Newell, 1979) was used to further define mortality in terms of thermal resistance lines (see later).

Animals were collected between September and December, 1984 and grouped into three size classes based on shell width (mm) which was measured as the greatest distance between the dorsal and ventral extremities of the shell. Juveniles were divided into two groups, namely <7 mm and between 7 mm and 35 mm, whilst breeding individuals were taken as >35 mm. This grouping was chosen as it corresponds

closely to the three major growth cohorts in the population (Cook & Birkett, 1986). The size-related intertidal distribution of *D. serra* also coincides with these size distinctions. The large animals occur subtidally while the smallest individuals are most abundant at mid-tide.

Forty bivalves from each size group were placed in 20-l tanks fitted with air-lift pumps which circulated sea water through a bed of sand. This ensured an even temperature throughout the water column and sand and thus individuals could not escape high temperatures by burrowing. The temperatures in the tanks ranged from 20°C to 38°C with increments of 2°C for a series of exposure times of 1, 3, 6, 12, 24, 48, 72 and 96 hrs. The upper temperature limit at which 50% of the bivalves retained an ability to burrow (BT_{50}) was also measured. Throughout the experiment, controls were maintained at 15°C and any incidental deaths in this group were used to correct data. Each observation allowed an assessment of the numbers of dead individuals, individuals which were buried and the number which were not buried but in a state of stress. Stress was evident from shell gaping and flaccid siphons and foot. For consistency, the same criteria for death used by Ansell *et al.* (1980a) and Ansell & McLachlan (1980) in studying *Donax* species were applied here, namely the absence of reaction to mechanical stimulation in the foot and siphons, of the mantle edge and adductor muscles. The absence of valve closure on stimulation of the cruciform muscle also proved a useful

criterion. If death was uncertain, possible recovery was monitored by placing an individual in fresh, circulating sea water at 15°C for 2 days.

Four individuals from each exposure temperature were transferred daily to 15°C in order to monitor recovery. The criterion for recovery was the ability of 50% of the test individuals thus transferred to burrow and ventilate after 4 days at 15°C.

Stepwise temperature exposure

These experiments were designed to establish whether stepwise exposure to the temperatures used above would improve survival rate. One hundred and twenty animals within each size group were progressively exposed to temperatures from 14°C to 36°C where the temperature was increased by 2°C each day over a total experimental period of 16 days. The sea water, circulated as before using air-lift pumps, was replaced every second day with fresh, well-aerated water at the appropriate temperature. Recovery was determined by transferring one individual to 15°C following 24 hrs of exposure to a test temperature after stepwise introduction to that temperature. The criterion for recovery was the same as used in the acute-temperature exposure experiments.

In this, and the above experiments, the median lethal temperature (LT_{50}) was determined graphically. The numbers dead, expressed as percentages of the original number, were plotted for each temperature and time interval and the

temperature at which 50% mortality occurred (median lethal temperature) was determined from the resulting plots. The temperature at which 50% of the bivalves originally present were buried, the median burial temperature (BT_{50}), was determined graphically in a similar manner. The same method has been used by a number of researchers (Bodoy & Masse, 1977, 1978; Ansell *et al.*, 1980a, b; Ansell & McLachlan, 1980). Probit analysis (Finney, 1964) enables a statistical measure of 50% lethal limits and this was applied to some of the data. This method provided similar or identical results, as found by Lent (1968), and since it is a long and tedious procedure, the graphical method described was used throughout.

The median lethal time was also determined graphically by plotting percentage mortality for each exposure temperature against the time of exposure on a logarithmic scale. In turn, the median lethal times were plotted as a function of exposure temperature on a semi-logarithmic scale to obtain thermal resistance lines for each size group; these lines define the zone of resistance of *Donax* (Newell, 1979).

Effect of temperature on valve movement and insulation

The effect of increasing temperature on valve movement and any possible insulation afforded by the shell was investigated by the simultaneous monitoring of temperatures inside the mantle cavity and the exterior. Unlike the experiments on temperature tolerances, sea water did not

circulate through the sand; thus there was a temperature gradient between the sand and water. This allowed a measure of the possible insulating benefits of the sand to burrowing individuals.

A fine hole was drilled through one valve close to the cruciform muscle in a total of 12 adults, 6 being used in each run. An iron-constantan thermocouple, calibrated within 0.1°C was inserted through the hole so that the tip came to lie deep within the mantle cavity and the hole was then sealed with dental wax. Attempts were made to enter the pericardial cavity to monitor temperature changes herein, but these always resulted in extreme stress or death of an individual.

The thermocouples inserted in the mantle cavity, plus another two, one hanging freely in water and the other embedded in sand, were connected to a data-logger which monitored temperatures continuously. Simultaneously, silver electrodes were cemented to each of the valves of a bivalve, distal to the umbo where the foot emerges and where maximum shell gape occurs. These electrodes were linked via a screened cable to an impedance pneumograph using D.C. coupling and then to an oscillograph which continuously monitored valve movement.

Animals were allowed to equilibrate for 24 hrs in a tank with well-aerated sea water at a constant temperature of 15°C. The presence of the thermocouples and electrodes did not impede burrowing and most animals buried themselves

completely. The temperature was increased to 20°C and then to 25°C, each increment taking 7 hrs. *Donax* were maintained at 25°C for 48 hrs and then the temperature of the cell was reduced to 15°C once again over a period of 12 hrs.

Effect of chlorine on survival

Three sources of chlorine can be used in toxicity studies, namely chlorine gas, sodium hypochlorite or calcium hypochlorite. Of these the last mentioned is recommended since it has the highest available free chlorine and is the most stable form of chlorine once dissolved in water (Burton, 1977). Therefore, even though chlorine is administered as sodium hypochlorite at the power station, calcium hypochlorite was used in the laboratory. It was dissolved in double distilled water rather than sea water because degradation is much slower in distilled water (Burton, 1977). A stock solution of 2 g calcium hypochlorite l^{-1} was made up daily in drip bags fitted with adjustable wheels whereby 24-hr dosage could be controlled by setting the number of drops per minute. Addition of chlorine in distilled water did not reduce salinity levels which were maintained between 34 and 35°/$_{oo}$ (see below). Since dosing with chlorine in this manner did not allow for precise control over concentration, it proved more practical to work within chlorine ranges.

The concentration of free residual chlorine in sea water was determined by a colour reaction with orthotolidine (Waugh, 1964; Bellanca & Bailey, 1977). This resulted in a

yellow colouration, the intensity of which could be compared to glass standards on a comparator disc ranging from 0.1 ppm to 2.00 ppm. Since chlorine toxicity is affected by salinity, pH and ammonium-nitrogen (Burton, 1977), these parameters were measured every second day. Salinity was adjusted to 34°/$_{oo}$ by diluting with double distilled water; pH was maintained at 7 and ammonium-nitrogen kept to a minimum by adding fresh sea water.

Five batches of 30 adult bivalves each were placed in separate 20-l tanks with slow flowing, aerated sea water at 15°C which was circulated through a bed of sand via air-lift pumps. Four batches were exposed to chlorine concentrations ranging from 0.1-0.3 ppm, 0.3-0.6 ppm, 0.6-0.9 ppm and 0.9-1.2 ppm for 2 weeks, whilst the fifth batch received no chlorine and served as a control. Each day the numbers of dead and buried *Donax* were recorded, and having corrected the data by taking into account deaths in the control tanks, median lethal times were calculated. Recovery was followed by transferring one individual from each chlorine level to a tank with fresh, flowing, non-chlorinated sea water every 24 hrs and noting the time taken to reburrow or die over 12 days.

HEART RATE EXPERIMENTS

Effect of temperature on heart rate

The electronic recording technique developed by Trueman (1967) was used to monitor heart rate. Two small holes were

drilled in each valve of the shell over the position of the heart. Fine silver-wire electrodes were inserted through these holes so that they lay on either side of the pericardium. The electrodes were sealed in place with dental wax and connected in series to an impedance pneumograph (A.C. coupling) and an oscillograph by a lightly screened cable. Changes in the impedance between the electrodes resulting from pulsatile changes in the volume of the heart or from movements of the animal within its shell were recorded on the oscillograph.

Adult bivalves with implanted electrodes, plus others without electrodes which served to demonstrate any detrimental effect of the implant on postural behaviour, were kept in 20-l tanks as described for the above chlorine experiments and allowed to equilibrate overnight at 15°C. As starvation can markedly depress heart rate in bivalves (Bayne, 1976), all animals were fed cultured algae during the equilibration period. Heart rate was initially monitored by transferring individuals directly from 15°C to either 20, 25 or 30°C for 1 to 2 days. However, this involved long periods of re-equilibration before recording could commence so as an alternative, the temperature was raised, without disturbing the animals, by 5°C every 24 hrs from 15°C to 30°C. Ultimately both methods of increasing temperature resulted in similar heart activity.

Preliminary experiments involving continuous recordings, indicated that no unpredictable short-term

variations in heart activity occurred. The heart rate of a single individual was, therefore, monitored for 15 mins in every hour for periods up to 18 hrs. The experiment was repeated 6 times at each temperature and 5 individuals were used per run, 4 with implants and 1 as a control.

One individual was removed daily from each exposure temperature and placed in fresh sea water at 15°C so that the rate of recovery over 24 hrs could be monitored. An equilibration period of 6 hrs was allowed before heart rate of these animals was measured.

Combined effect of temperature and chlorine on heart rate

Adult bivalves were simultaneously exposed to the temperature and chlorine concentration regime described above. Experiments were repeated 3 times for each chlorine range and temperature using 5 individuals per run in which one served as a control without implanted electrodes. As before, some individuals were removed daily after 24 hrs exposure to each treatment, placed in non-chlorinated sea water at 15°C and allowed to equilibrate for 6 hrs before monitoring the recovery heart rate for 24 hrs.

RESULTS

SURVIVAL EXPERIMENTS

Acute temperature exposure

An example of the graphical determination of LT_{50} values, that is the temperatures at which 50% mortality is predicted after acute exposure to near lethal temperatures, is given in Fig. 2.1a for the size group >7 <35 mm. LT_{50}'s thus obtained were plotted against the exposure time in Fig. 2.1b for all *Donax* sizes used in the experiments. These plots show that during the first 12 hrs LT_{50}'s fell from 37°C/38°C to 34°C and after 24 hrs to between 31°C and 33°C for all *Donax*. The rapid decline in LT_{50} values within the first 24 hrs indicates the extreme sensitivity of the bivalves to temperatures above 31°C, where a 2°C increase sometimes meant the difference between zero and 100% mortality. From 24 to 96 hrs, when the experiment ended, a clear distinction emerged between the tolerance limits of the three size groups, a stable LT_{50} of 30°C being reached after 48 hrs for the small ones, but only occurring after 72 hrs at 28°C for individuals >7 <35 mm and at 27°C for large *Donax* (Fig. 2.1b). Thus the smallest showed marginally better survival of acute temperature exposure resulting in a steady LT_{50} value 3°C higher than for the largest animals after 4 days.

Median lethal times were estimated from plots such as that given in Fig. 2.2a in which percentage mortality is a

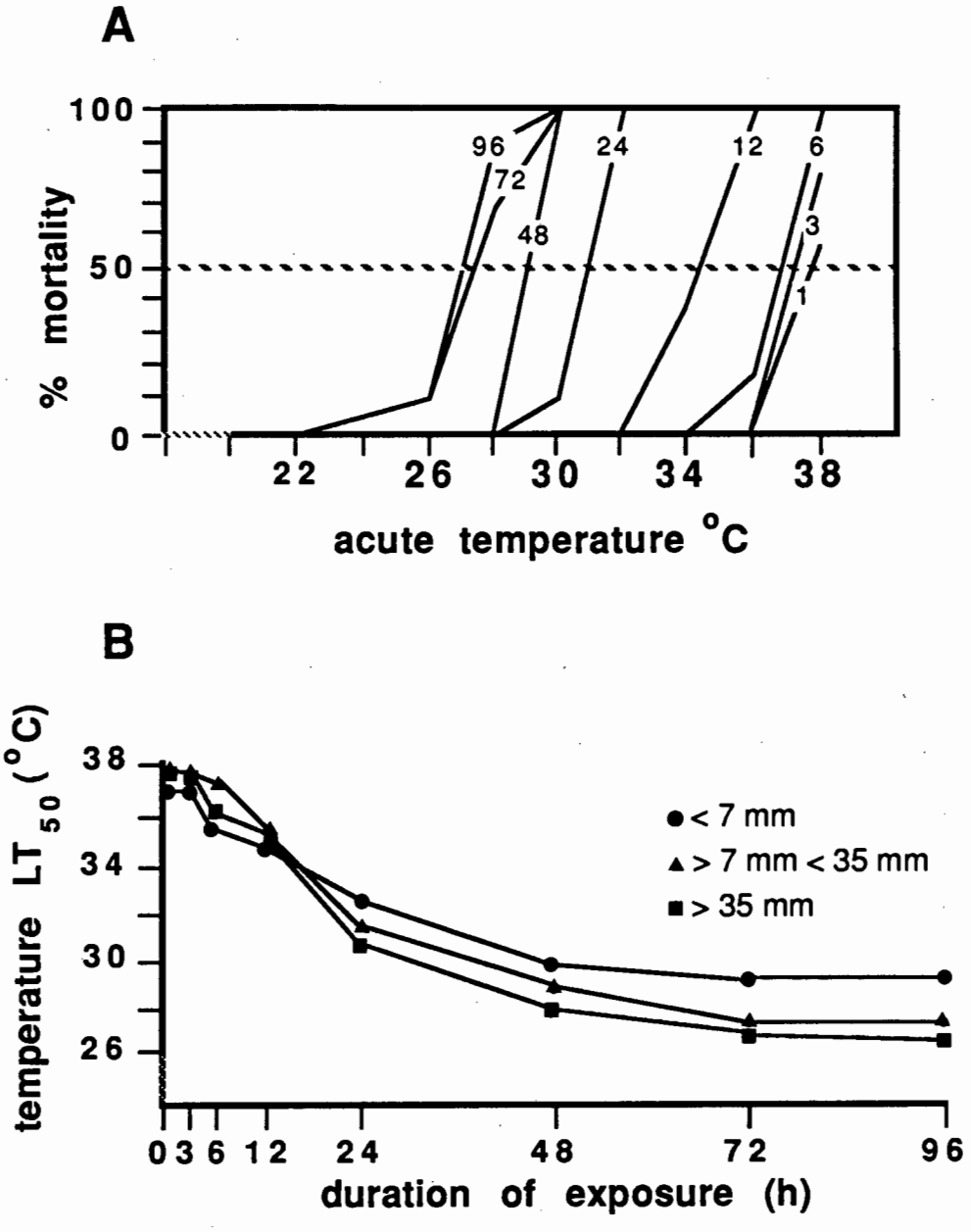

Fig. 2.1. Estimation of the median lethal temperature (LT_{50}) for the size group >7 <35 mm acutely exposed to temperatures ranging from 20 - 38 °C for time intervals between 1 and 96 hrs (**A**). Resultant LT_{50}'s are plotted against exposure time in three size groups of *D. serra* (**B**).

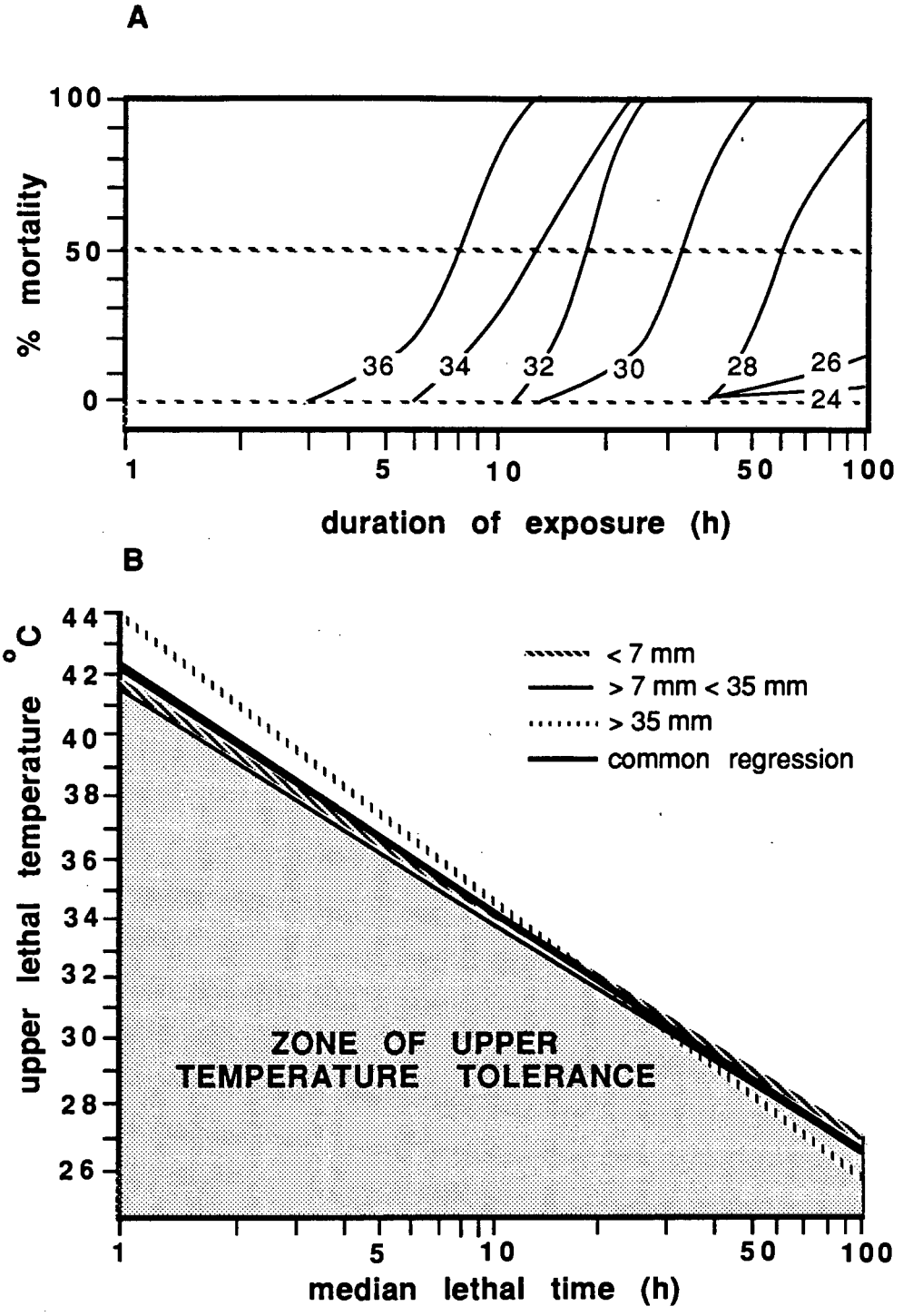

Fig. 2.2. Cumulative percentage mortality of sizes > 7 mm < 35 mm against exposure time at two degree intervals from 24 to 36 °C (A). Resultant median lethal times are plotted against upper lethal temperatures in B for all sizes. Data are fitted to linear regressions on semi-logarithmic axes to produce thermal resistance lines. The common regression designates the zone of upper thermal tolerance (▭).

function of time on a semi-logarithmic scale for middle-sizes. These median times were logarithmically regressed against upper lethal temperatures in Fig. 2.2b so producing thermal resistance lines which define the zone of thermal tolerance. It is evident that the survival time for all *Donax* shows an exponential decline as exposure temperature increases. The difference in the three slopes indicates that at temperatures above 30°C, the middle size group displayed the best thermal resistance whereas below this temperature, the smallest individuals displayed the highest upper temperature tolerance. These trends, although not as clear, were also evident in the LT_{50} values in Fig. 2.1b. However, differences relating to size can only be regarded as marginal, since there is no significant difference between the resistance lines in Fig. 2.2b ($P<0.05$). All data can therefore be combined to produce a single zone of tolerance for all *Donax* where the resistance line (Y) = 42.02 - 7.72(logX). With extrapolation this line defines the upper lethal temperature as 42°C and the incipient temperature as 25°C (= highest temperature to which an organism can be continuously exposed for an indefinite period without increasing mortality).

An example of the graphical estimation of temperatures at which 50% of bivalves >7 <35 mm remained buried (BT_{50}) is shown in Fig.2.3a and the resultant plots of BT_{50} values against duration of exposure appear in Fig. 2.3b for all sizes. During the first hour there was a distinct size-

related difference; the smallest *D. serra* burrowed immediately on transference to temperatures near the lethal limit (*viz.* 36°C and 38°C), whereas the much lower BT_{50} values for large sizes reflected a delay in burrowing following thermal shock (Fig. 2.3b). However, during the next 11 hrs stressed small to medium sized *D. serra* re-emerged prior to becoming thermally paralysed and dying, resulting in a drastic drop in BT_{50}'s from 36/37°C to 30°C. For large animals on the other hand, BT_{50}'s increased slightly as a consequence of many animals on the sand surface burying themselves after initial thermal shock. Between 12 and 48 hrs, BT_{50} values stabilised for all sizes, 50% of the larger animals remaining buried at temperatures 2-3°C higher than individuals <35 mm. Over the last 2 days of the experiment, values remained steady for the smallest bivalves, but declined to 26°C for the larger ones as they gradually emerged from the sand to lie on the surface with their shells gaping. At this point BT_{50} and LT_{50} values were nearly equal for each respective size group, emphasising the close association between emergence from the sand and death due to thermal stress (Figs. 2.1b & 2.3b).

In Fig. 2.4, recovery of test individuals is defined as the ability of 50% of animals to burrow and ventilate after being returned to 15°C for 4 days from temperatures between 20°C and 38°C. Based on these criteria, all sizes of *Donax* recovered after 4 days exposure to temperatures between 20°C and 28°C and after 1 day at 30°C. Only large individuals

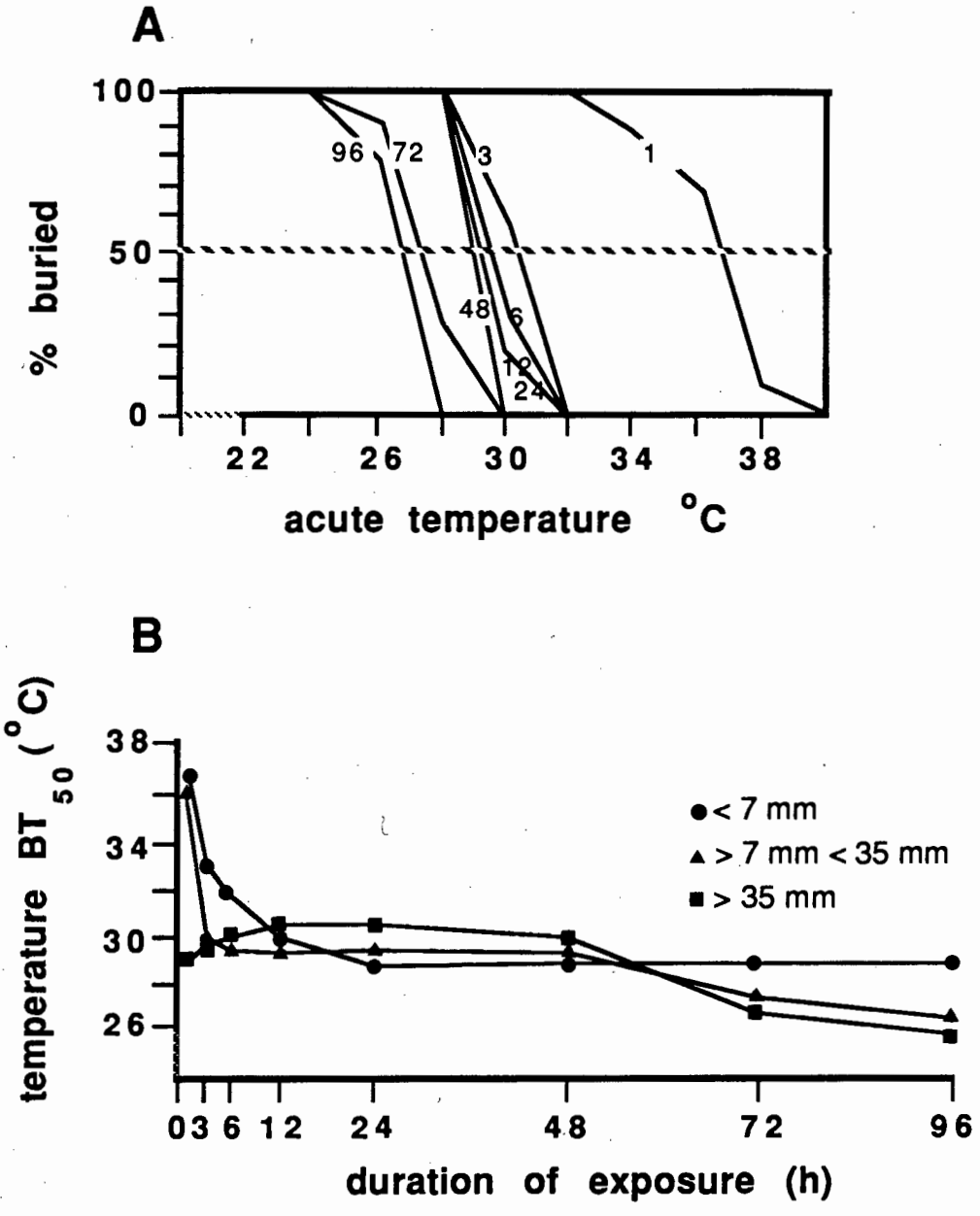

Fig. 2.3. Estimation of the median burial temperature (BT_{50}) for the size group >7 <35 mm acutely exposed to temperatures ranging from 20 - 38 °C for time intervals between 1 and 96 hrs (**A**). Resultant BT_{50}'s are plotted against exposure time in three size groups of *D. serra* (**B**).

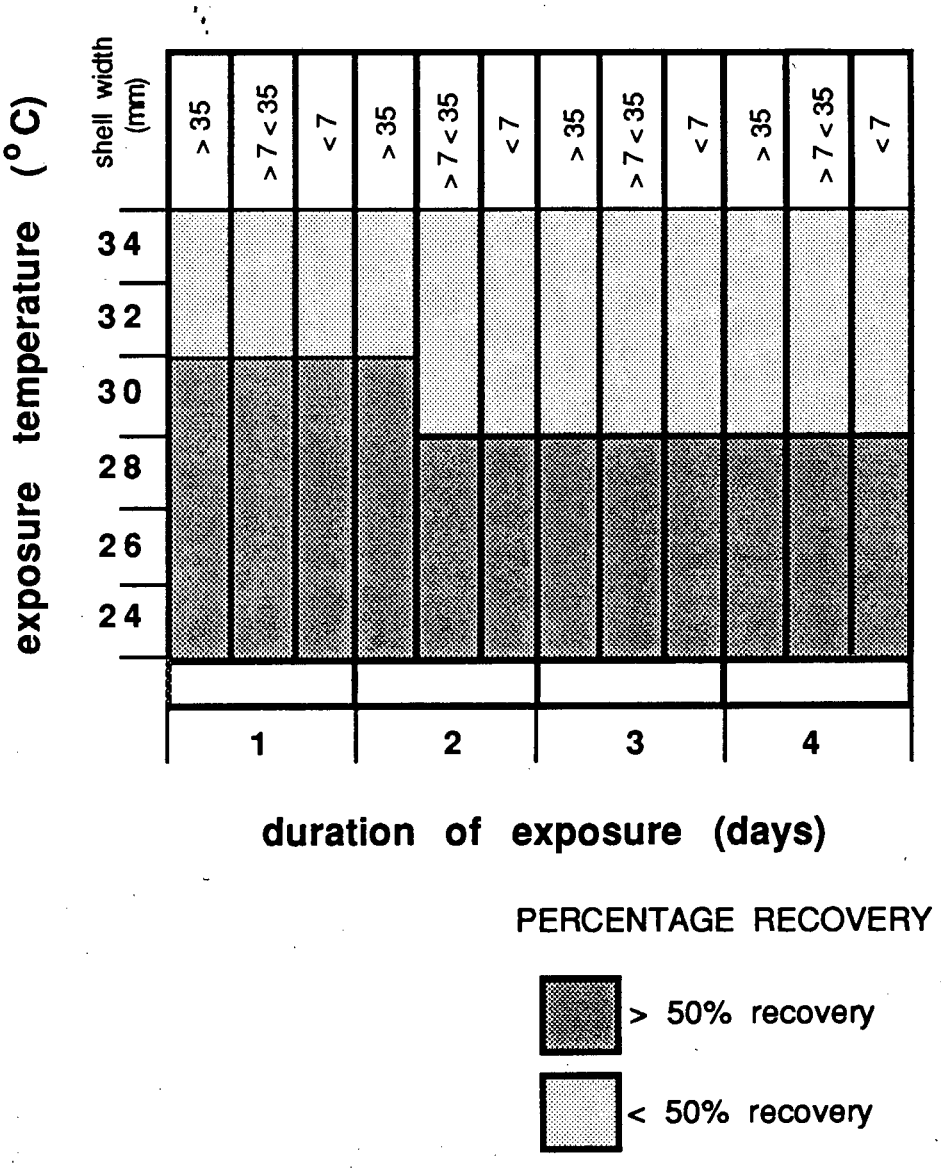

Fig. 2.4. Recovery rate over 4 days at 15 °C following acute exposure for 4 days to temperatures ranging from 20 - 38 °C for 3 size groups of *D. serra*.

recovered after 2 days at 30°C, whereas more than 50% of medium and small sizes lay on the sand surface with their shells gaping and eventually died. No further recovery was observed for any size beyond 2 days exposure to 30°C, and at 32°C and above, there was no recovery, even after only 1 day exposure.

Stepwise temperature exposure

In these experiments, designed to investigate the possibility of improving survival rate by gradually exposing *D. serra* to high temperature, LT_{50}'s were determined graphically (Fig. 2.5a) in a similar manner to Fig. 2.1b. A difference lies in that for any one particular temperature, the percentage mortality was plotted for the number of days, not hours, exposed to that temperature **after** the temperature was reached by stepwise increments of 2°C per day from 14°C. All bivalves <7 mm held at 36°C for 1 day (after stepwise introduction) died, whereas for individuals >7 mm this occurred at 32°C, resulting in corresponding LT_{50} values of 33°C for the small size and 30°C for the larger ones (Fig. 2.5b). The decline in LT_{50}'s from these values over 4 days was similar to that for acute exposure for the same duration (Fig. 2.1b), values being only slightly higher by 1 to 2°C. This similarity demonstrates that stepwise exposure to increasing temperatures employed in these experiments did little to enhance survival.

As exposure increased to 12 days, the temperatures at which 50% mortality occurred remained fairly constant,

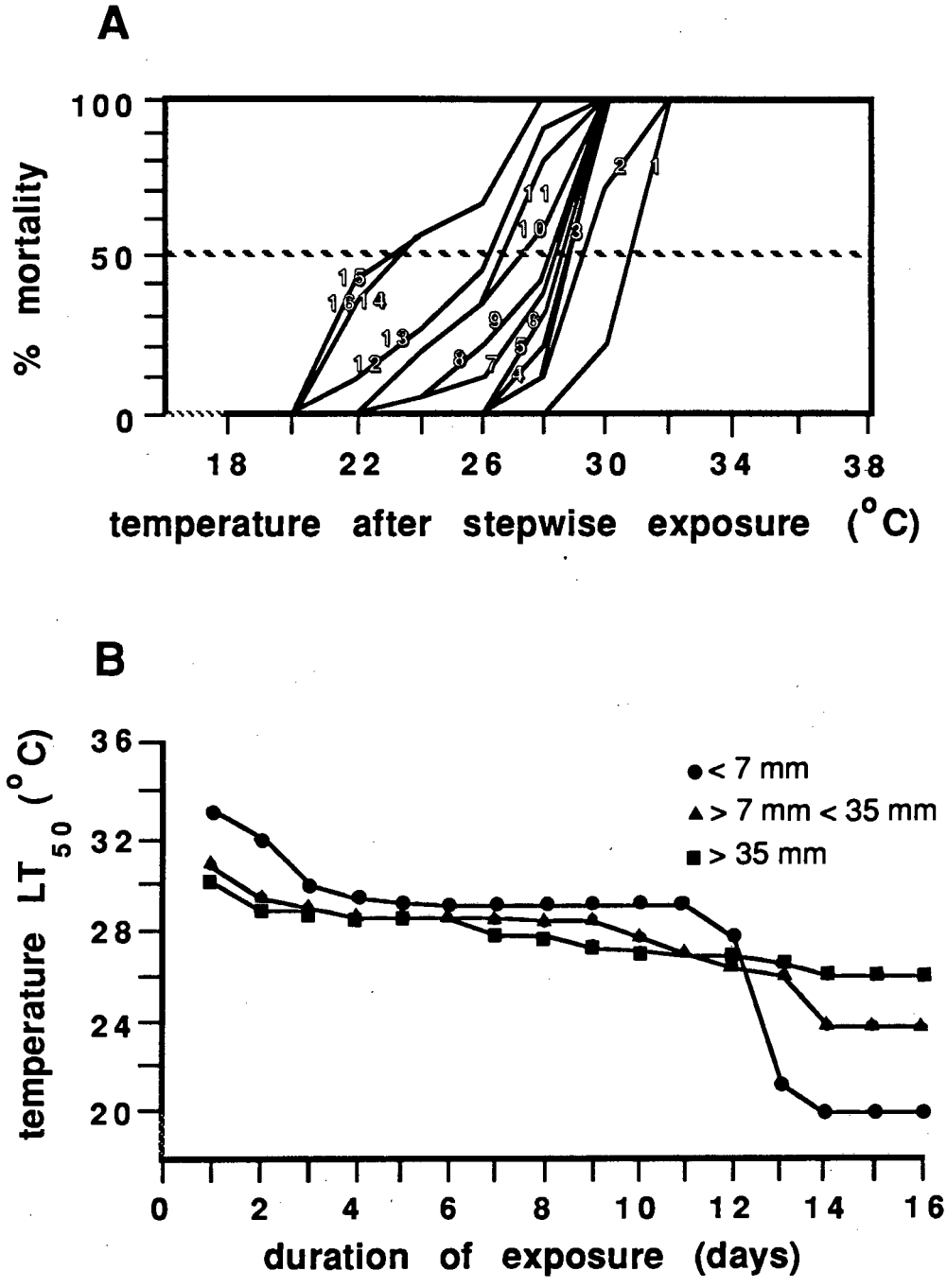

Fig. 2.5. Estimation of the median lethal temperature (LT_{50}) for the size group >7 <35 mm gradually exposed to temperatures increased by 2 °C per day from 14 - 38 °C over a period of 16 days (**A**). Resultant LT_{50}'s are plotted against exposure time in three size groups of *D. serra* (**B**).

between 27°C and 30°C for all sizes. For small *Donax*, longer exposure resulted in LT_{50} declining rapidly to 20°C and then stabilising from 14 to 16 days of exposure. LT_{50}'s for larger bivalves followed the same trend, but stability was reached at higher LT_{50} values; 24°C for individuals >7 <35 mm and 26°C for those >35 mm. Unlike acute exposure, stepwise exposure up to 16 days resulted in large bivalves displaying the better thermal tolerance.

BT_{50}'s were estimated from plots such as that given in Fig. 2.6a for bivalves >7 <35 mm and appear against duration of exposure in Fig. 2.6b; values closely followed LT_{50}'s for the first 8 days of exposure (Fig. 2.5b) when individuals died as soon as they emerged from the sand. Once again a slight increase in BT_{50} for large sizes during the first 2 days of exposure represents an initial delay in burrowing, even though the animals had gradually experienced increasing temperatures so that thermal shock was not as pronounced as in acute exposure. After being held at test temperatures for more than 8 days, BT_{50}'s lagged behind LT_{50} values as more and more individuals emerged from the sand to lie on the surface before dying. By the end of the experiment $BT_{50} = LT_{50}$ for small *Donax*, meaning that 50% of the bivalves emerged and died at the same median temperature of 20°C. For larger animals BT_{50} = 23°C, whereas LT_{50} = 26°C, the difference demonstrating that in this size group, individuals surface at a temperature below the 50% lethal limit and lie gaping for some time before dying.

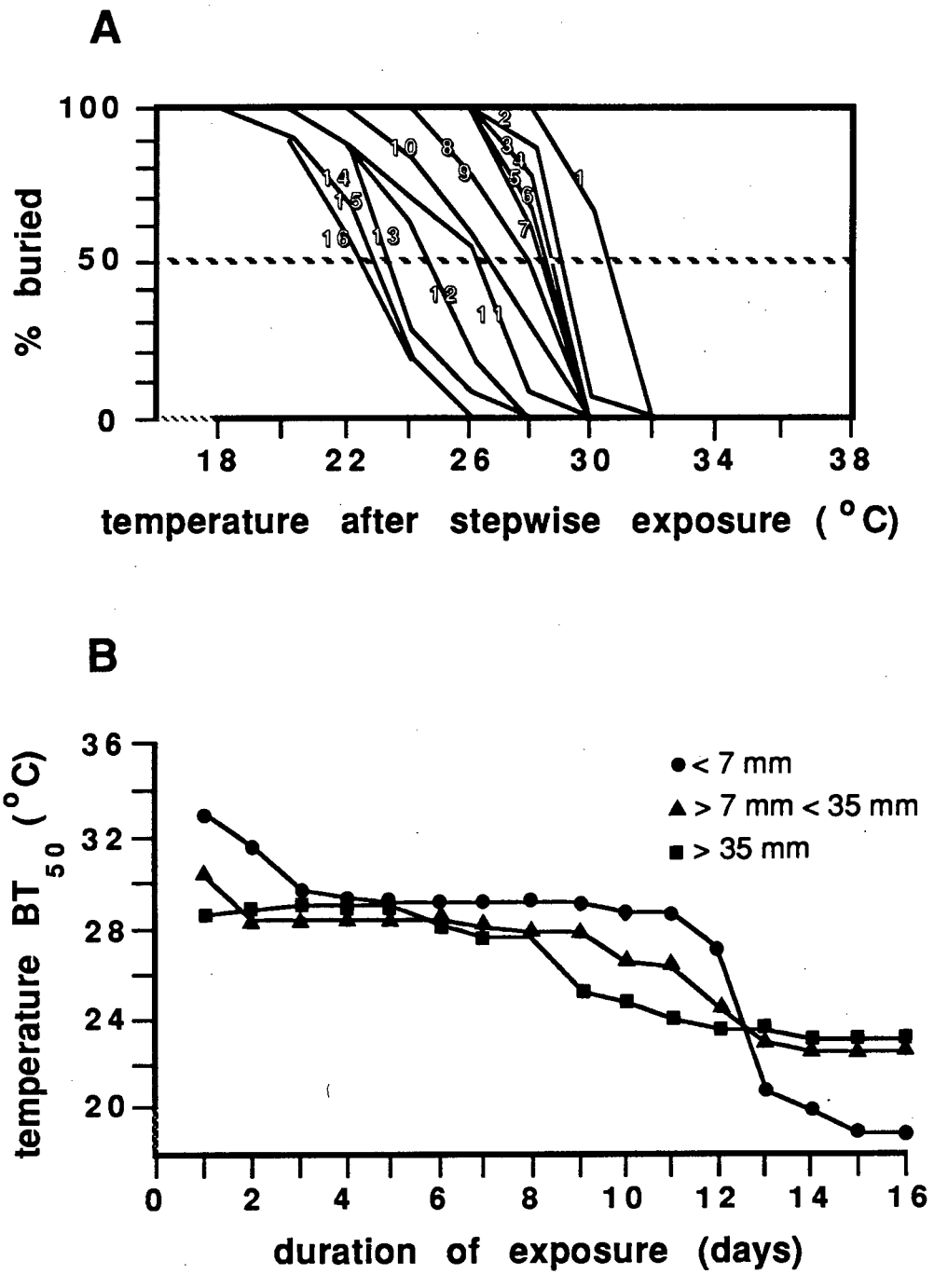

Fig. 2.6. Estimation of the median burial temperature (BT_{50}) for the size group >7 <35 mm gradually exposed to temperatures increased by 2 °C per day from 14 - 38 °C over a period of 16 days (**A**). Resultant BT_{50}'s are plotted against exposure time in three size groups of *D. serra* (**B**).

Recovery after stepwise exposure to temperatures from 14°C to 36°C is shown in Fig. 2.7. More than 50% of all sizes recovered after 16 days exposure to temperatures less than and equal to 26°C, after 13 days at 28°C and after 1 day exposure to 30°C. Following 14 and 15 days at 28°C, only large individuals recovered and after 16 days more than 50% of all sizes showed no signs of recovery. At 30°C, following 2 days exposure, medium and small sizes recovered, but after 3 and 4 days only small ones fulfilled the recovery criteria; beyond 4 days no recovery was noted. At 32°C large *Donax* recovered after 1 day, but beyond this time and at higher temperatures all sizes lay on the surface until death.

Valve movement and shell insulation

During equilibration at 15°C, pronounced valve adductions (ca. 1 min^{-1}), were monitored while individuals ventilated and burrowed (Fig. 2.8). For the next 7 hrs as temperature was raised to 20°C only faint valve movements associated with repositioning in the sand were visible, but unfortunately these were too weak to be detected by the pneumograph. The bivalves remained buried with their siphons open and shells gaping slightly. Since in this position ventilation continued, the temperature in the mantle cavity naturally paralleled any increase in the surrounding water.

During the increase to 25°C, shell gape increased slowly without noticeable adductions and both siphons became

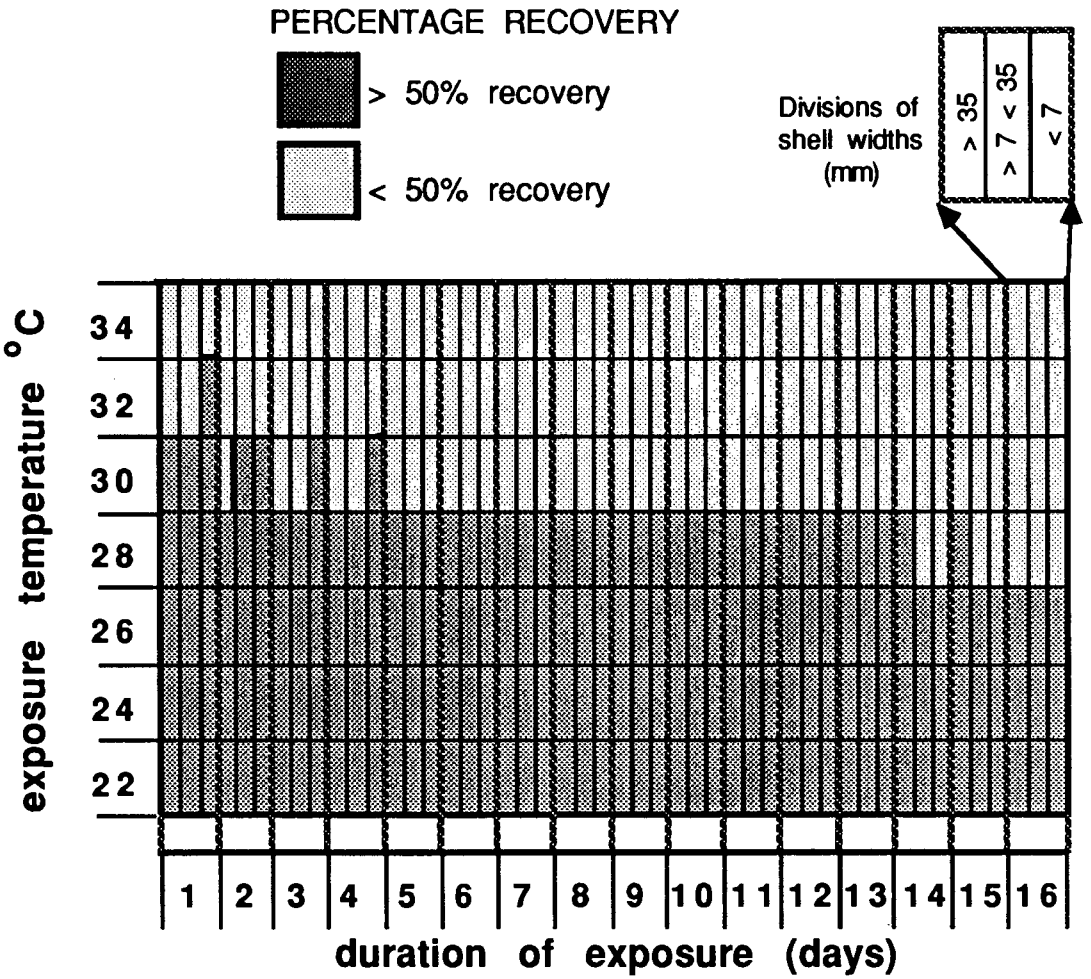

Fig. 2.7. Recovery rates after 4 days at 15 °C following stepwise exposure at 2 °C per day to temperatures ranging from 14 - 38 °C for all three size groups of *D. serra*.

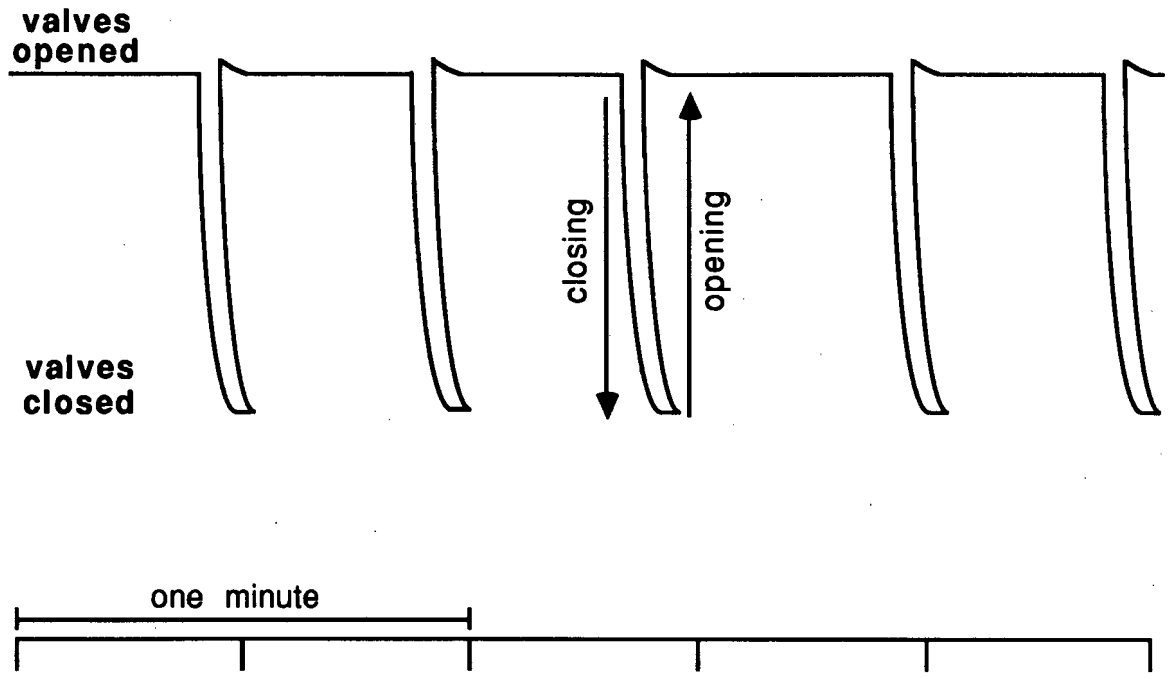

Fig. 2.8. Trace depicting numerous valve adductions associated with burrowing and ventilating in an adult individual at 15 °C (trace monitored on DC current).

fully extended. Three different behavioural responses, probably related to natural variability in the condition of individuals, were noted after 40 hrs at 25°C. Approximately 20% of the test individuals emerged from the sand, whereafter their siphons became limp and valves gaped further until the cruciform muscle was clearly visible and taut. Bivalves in this condition never survived, even when the temperature was gradually lowered to 15°C over 7 hrs. Most (65%) remained buried and extended their siphons further, retaining a small shell gape until the temperature was returned to 15°C when valve movement was once again evident on the oscillograph. In both these behavioural responses the temperature in the mantle cavity equalled that outside. In the third response, approximately 15% of the bivalves withdrew their siphons and foot and remained as deep as possible in the sand with their valves closed. In this position, the shell, together with the surrounding sand, afforded some insulation; the mantle cavity temperature was 1°C to 2°C below the surrounding water. However, this position, besides being the least common response, was seldom retained until the end of exposure to 25°C; thus any insulation only occurred for periods under 40 hrs. In these individuals the siphons eventually extended once again, accompanied by shell gape, but on return to 15°C, the siphons became shorter and the gape decreased. It should be noted, however, that sand was provided to a depth

of 15 cm only and in the field adults can be found at 30 cm, a depth which could provide significantly better insulation.

Effect of chlorine on survival

Effect of chlorine in the range 0.1 - 1.2 ppm on percentage mortality and burial response in adult *D. serra* at 15°C is illustrated in Fig. 2.9. On dosing at all concentrations *Donax* immediately retracted the siphons and foot and closed the valves while buried in the sand. At the lowest chlorine concentration this position was maintained intermittently for approximately 3 - 6 hrs whereafter the valves and siphons remained open. After 14 days exposure, no adverse effects were apparent; mortality was zero and all individuals remained completely buried with their siphons fully or partially open on the surface.

Following initial dosing between 0.3 and 0.6 ppm, animals remained retracted for 1 to 2 days and then periodically opened and closed their valves and siphons to ventilate over the next 12 days; a low % mortality (<10%) was preceded by emergence of approximately 30% of individuals. No median lethal times were measurable below 0.6 ppm.

At concentrations above 0.6 ppm, animals remained withdrawn with their siphons and valves tightly closed for 7 to 8 days during which time internal tissues were effectively isolated and thus protected from the external medium. Hereafter shell gape increased and the siphons became limp and eventually all emerged from the sand to lie

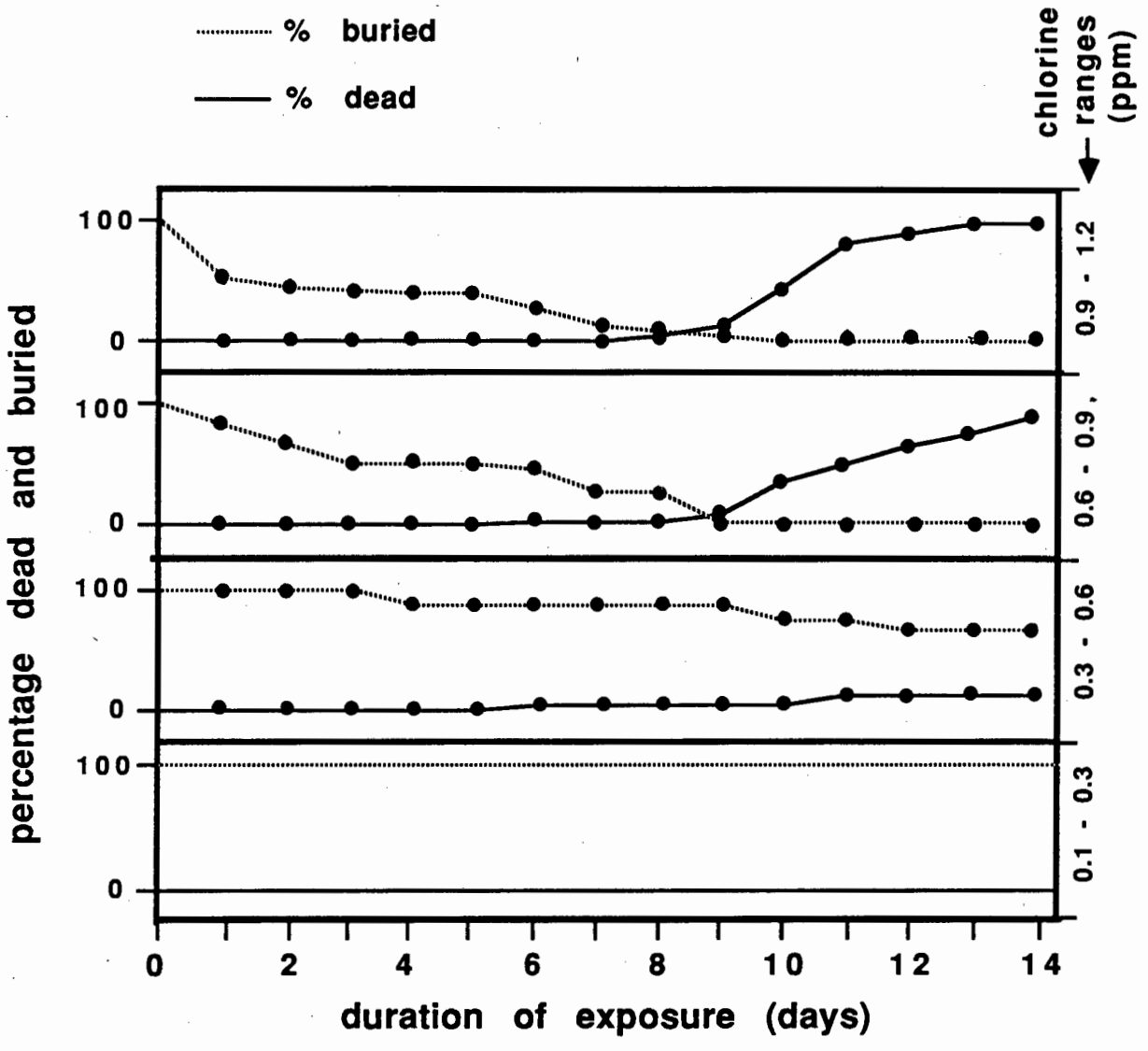

Fig. 2.9. Effect of chlorine in the range 0.1 - 1.2 ppm on the mortality rate and burial response of *D. serra* at 15°C.

on the surface with their shells wide open. The median lethal time for the range 0.6 - 0.9 ppm was 10.5 days and between 0.9 and 1.2 ppm it was 10 days. By the end of the 14 day experiment 90% of those exposed to 0.6 - 0.9 ppm were dead, as were 100% at 0.9 - 1.2 ppm.

The recovery of individuals removed daily from the experiment and placed in non-chlorinated sea water for 12 days is depicted in Fig. 2.10. There was full recovery of bivalves following 6 days exposure to all concentrations of chlorine, although some delay in re-burrowing was observed in the range 0.6 to 1.2 ppm. However, beyond the sixth exposure day, >50% of individuals subjected to >0.6 ppm never re-buried in fresh sea water. Time on the surface before dying ranged from 5 days, after 7 days exposure, to less than one day by the end of the experiment. On the other hand, at concentrations <0.6 ppm, >50% of transferred animals recovered fully, although re-burial was slow after 12 days exposure.

HEART RATE EXPERIMENTS

Effect of temperature

The basal heart rate fluctuated between 11 & 15 beats min^{-1} at 15°C. While buried and ventilating with a slight valve gape and siphons open on the surface, heart activity steadied at 13 $\pm$ 2 beats min^{-1} and experiments only commenced once this rate was maintained for at least 24 hrs (Fig. 2.11). When the temperature was raised to 20°C, beat

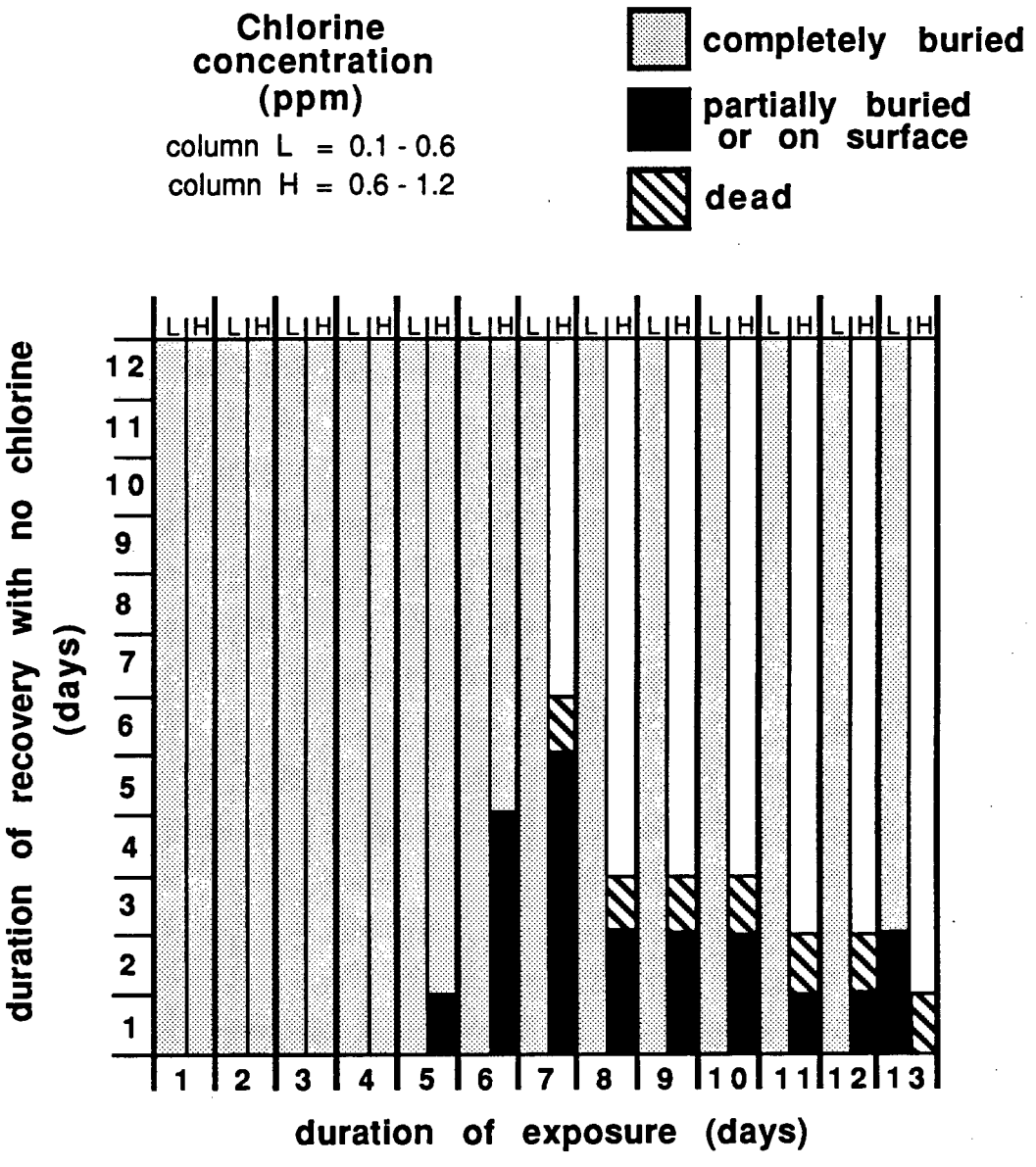

Fig. 2.10. Recovery of adult individuals at 15 °C with no chlorine following exposure to chlorine in the range 0.1 - 0.6 and 0.6 - 1.2 ppm. Total recovery was recognised by an ability to reburrow (□), partial recovery by an inability to burrow (■) and no recovery by the ultimate death of individuals (▨). Recovery criteria were set by the relevant behaviour of greater than 50% of the animals.

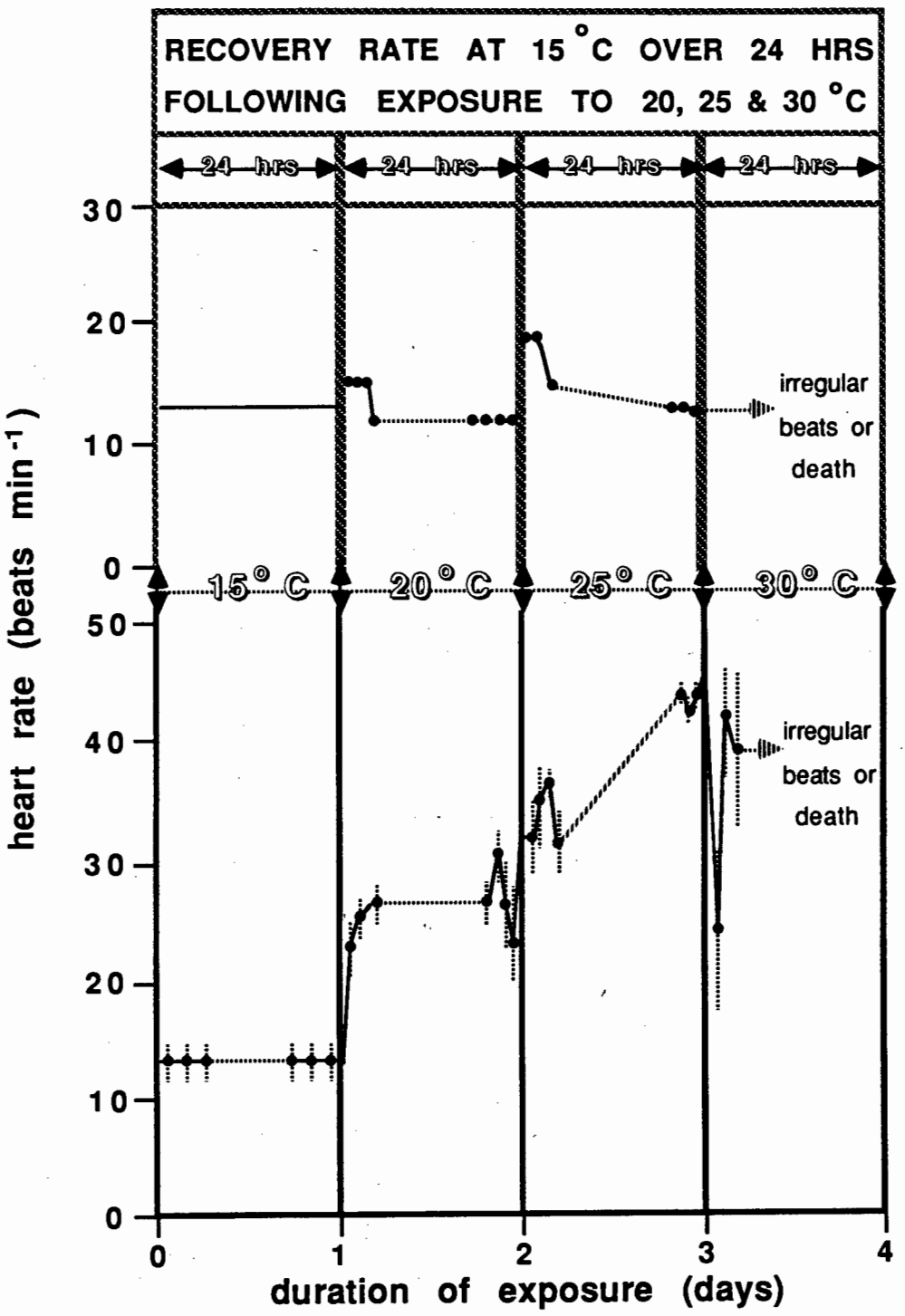

Fig. 2.11. Effect of temperature on heart rate of adult individuals when raised 5 °C per day from 15 to 30 °C. Recovery after 24 hrs exposure to each test temperature was followed for 24 hrs at 15 °C following a 3 hr settlement period. Vertical bars (|) represent one standard deviation above and below the mean.

frequency increased immediately, although the maximum rate of 26 beats min^{-1} was only reached after 5 hrs. After 24 hrs at 20°C and at the beginning of exposure to 25°C, heart activity became less stable, fluctuating between means of 18 and 33 beats min^{-1} with standard deviations much higher than for data at 15 or 20°C. Just before the temperature was raised from 25°C to 30°C, heart rate had risen to 44 beats min^{-1}, but at 30°C it oscillated wildly and animals were either near death or dead after 12 hrs exposure. Since individuals retained as controls (without electrodes) showed extreme stress but did not die at 30°C, death of experimental animals at this temperature must to some degree be attributed to the presence of the implanted electrode. When maximum beat frequencies are considered, Q_{10} values equalled 4.0 between 15 and 20°C and 2.9 from 20 to 25°C.

The recovery of individuals, following 3 hrs equilibration on transference to 15°C after 24 hrs exposure to test temperatures is also shown in Fig. 2.11. A equilibration time was necessary before monitoring began to allow beat frequencies to stabilise after an overshoot and irregularity in heart rate following direct transference. Those exposed to 20 and 25°C recovered completely as shown by the return to the normal beat frequency of $\pm$13 min^{-1}. However, bivalves from 30°C continued to display erratic heart rates and after monitoring for 24 hrs at 15°C, were either dead or lying on the surface with the shell gaping widely and still displaying irregular beats. Although no

controls died during the recovery test, some also came to lie stressed on the surface, indicating that it is temperature and not the electrodes which initiated such a response.

During this experiment, some interesting responses to increasing temperature were suggested by the traces of heart activity. At 15°C the basal beat was often interrupted by numerous adductions as *Donax* burrowed deeply into the sand (Fig. 2.12a). As the temperature was raised to 20°C, frequency increased rapidly from 15 to 22 beats min^{-1} in 5 minutes (Fig. 2.12b); adductions were less frequent, more protracted and often followed immediately by cessation of heart beat for about 30 seconds when the valves closed (Fig. 2.12c).

At 25°C regular beating often gave way to periods of extensive contraction and retardation or suppression (Fig. 2.12d). Periods of suppression often alternated with periods of reduced beat amplitude and this coincided with the brief withdrawal of siphons followed by rapid re-extension and commencement of ventilation (Fig. 2.12e). Throughout exposure to 25°C, the shell gaped to varying degrees, and the siphons were fully extended most of the time.

Shell gape was maximal at 30°C and both siphons remained fully extended but very limp. Beat frequency, although rapid and often steady, was of very low and irregular amplitude with infrequent adductions, followed by

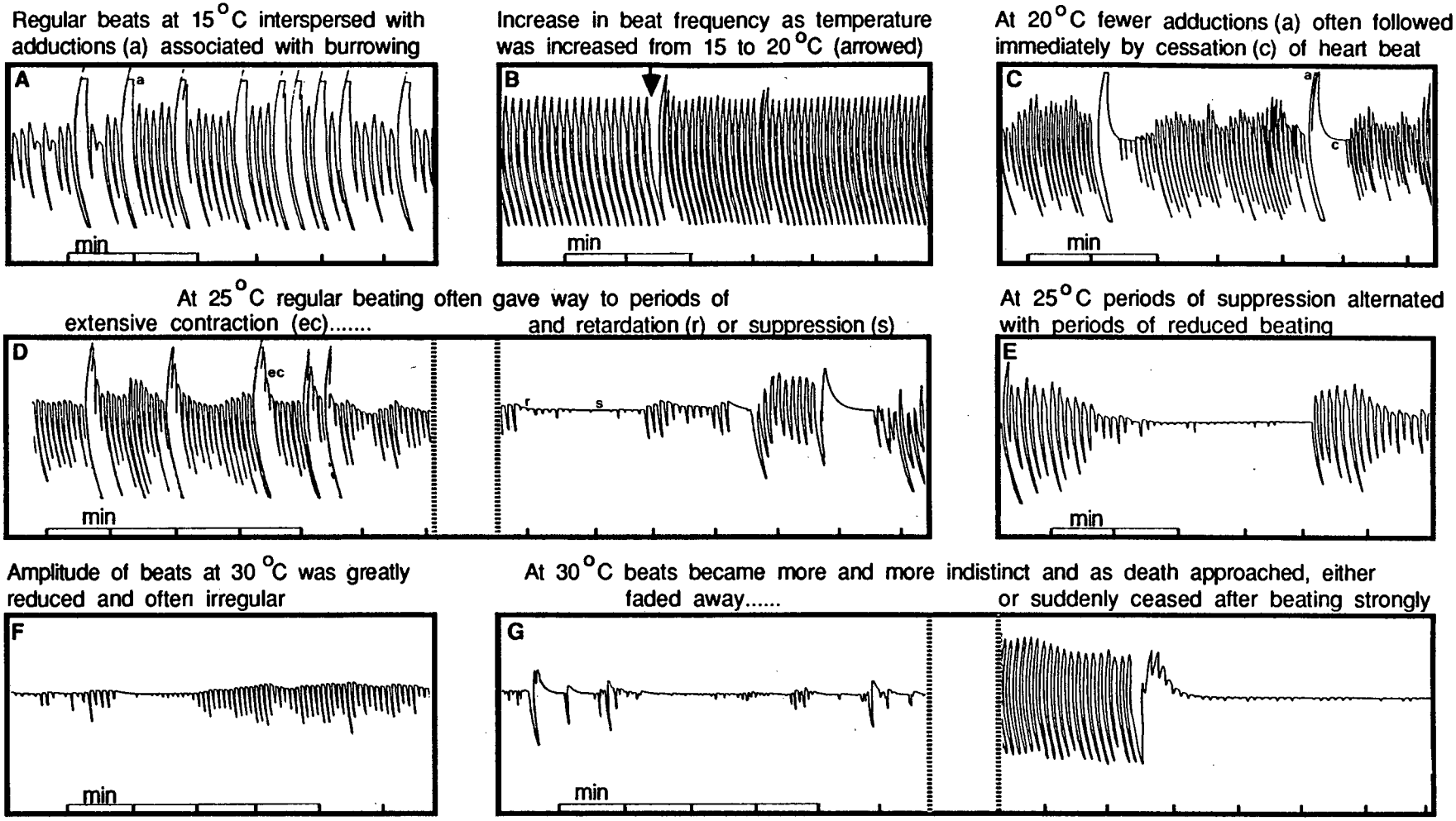

Fig. 2.12. Effect of temperature on the heart rate of adult individuals using the impedance technique with AC coupling.

an extended suppression of heart activity (Fig. 2.12f). At death the shell remained gaping, the siphons collapsed and beats became more and more indistinct, fading away or ceasing after a period of regular, strong beats (Fig. 2.12g).

Combined effect of chlorine and temperature

Within the chlorine range of 0.1 to 0.3 ppm at 15°C, beat frequency dropped immediately on dosing from the basal rate of 13 min^{-1} to a mean of 8 and then 7 min^{-1} (Fig. 2.13), corresponding to withdrawal of the animal and valve closure as observed in the earlier chlorine experiment. An increase of heart rate to 10 beats after 24 hrs exposure at 15°C coincided with a gradual re-emergence of the animal. On raising the temperature to 20°C and then 25°C over the next 48 hrs, beats slowly increased to 11 and 14 min^{-1} respectively. This frequency is nearly 4 times lower than that reached at 25°C in the absence of chlorine (see Fig. 2.11). During this period the valves gaped slightly and the siphons stayed open but did not extend. At 30°C, 13 to 14 beats min^{-1} were maintained for 6 hrs and then heart activity became indistinct as animals began dying. When dosed with chlorine in the range 0.3 to 0.6 ppm at 15°C, heart rate dropped to 7 and then 5 beats min^{-1} and only rose to 10 min^{-1} at 20°C when the valves began to gape a little. At 25 and 30°C the heart responded as for the dosage of <0.3 ppm (Fig. 2.13).

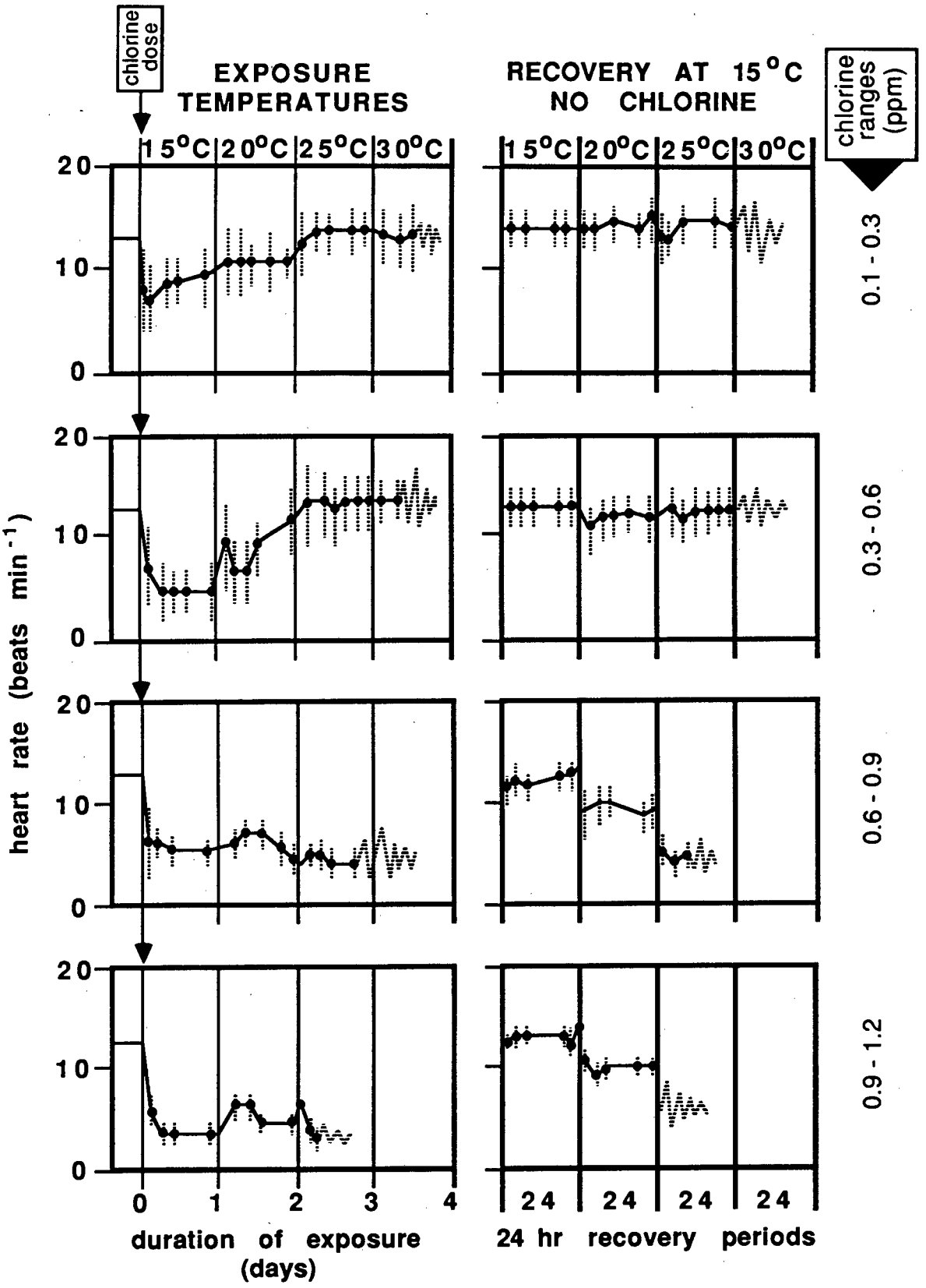

Fig. 2.13. Combined effect of temperature and chlorine on the heart rate of adult individuals over 4 days with a 5 °C increment each day. Recovery from exposure to each test temperature and chlorine range was monitored for 24 hrs after allowing 3 hrs for settlement on transference to 15 °C.

Between 0.6 and 0.9 ppm, beat frequency also decreased to 5 min^{-1} on dosing at 15°C, but never rose above 7 min^{-1} over the next 2 days as the temperature was increased from 20°C to 25°C (Fig. 2.13). After 18 hrs at 25°C, the heart beat showed no regular pattern and bivalves began dying once the temperature reached 30°C. A similar response occurred in the range 0.9 to 1.2 ppm, when heart rate never rose above 6 beats min^{-1}, becoming indistinct after 3 hrs at 25°C; individuals began dying before the temperature was raised to 30°C. As before the animals withdrew their siphons and feet and closed the valves on initial dosing, but as the temperature increased, the valves gaped more and more, thereby directly exposing the pallial organs to high levels of chlorine. At no time were the siphons re-extended and the foot only emerged from the gaping valves on paralysis.

Q_{10} was negative above 0.6 ppm, demonstrating that the effect of chlorine at these concentrations predominated so that heart rate was no longer positively temperature dependent. In addition, heart activity was suppressed to a steady slow beat with small standard deviations from means irrespective of temperature, whereas at concentrations below 0.6 ppm, deviations were higher and more variable as both temperature and chlorine interacted to raise and suppress beat frequency (Fig. 2.13).

After allowing 3 hrs for equilibration on transference to non-chlorinated sea water at 15°C, all specimens exposed to chlorine <0.6 ppm and a temperature of 25°C and less

recovered fully as beat frequency returned to the normal basal rate of 13 min^{-1} (Fig. 2.13). For the equivalent 24 hr observation period, individuals from 30°C either died or continued to display irregular heart activity, with no indication of recovery. In the range 0.6 to 1.2 ppm, a return to normal beat frequency in fresh sea water was only observed in individuals from 15°C (Fig. 2.13). Individuals exposed to 20°C displayed a suppressed frequency (10 beats min^{-1}) during the time heart rate was monitored, whereas those from 25°C retained the disrupted pattern observed while in chlorinated sea water and those from 30°C died.

The patterns of heart activity illustrated by these experiments provided some interesting contrasts to those obtained when investigating the effect of temperature alone. After dosing with chlorine between 0.1 and 0.6 ppm, heart beats became greatly protracted (Fig. 2.14a), but once the temperature was raised to 20°C, beats began increasing towards the basal rate (Fig. 2.14b). At 25°C, beats were once again protracted but weaker with periods of total suppression (Fig. 2.14c) and at 30°C, they were even weaker and hence lower in amplitude and indistinct (Fig. 2.14d). Above 0.6 ppm, beats became irregular on dosing at 15°C and this pattern persisted at 20°C (Fig. 2.14e). At 25°C, regular protracted beats disintegrated into an indiscreet pattern as animals neared death (Fig. 2.14f).

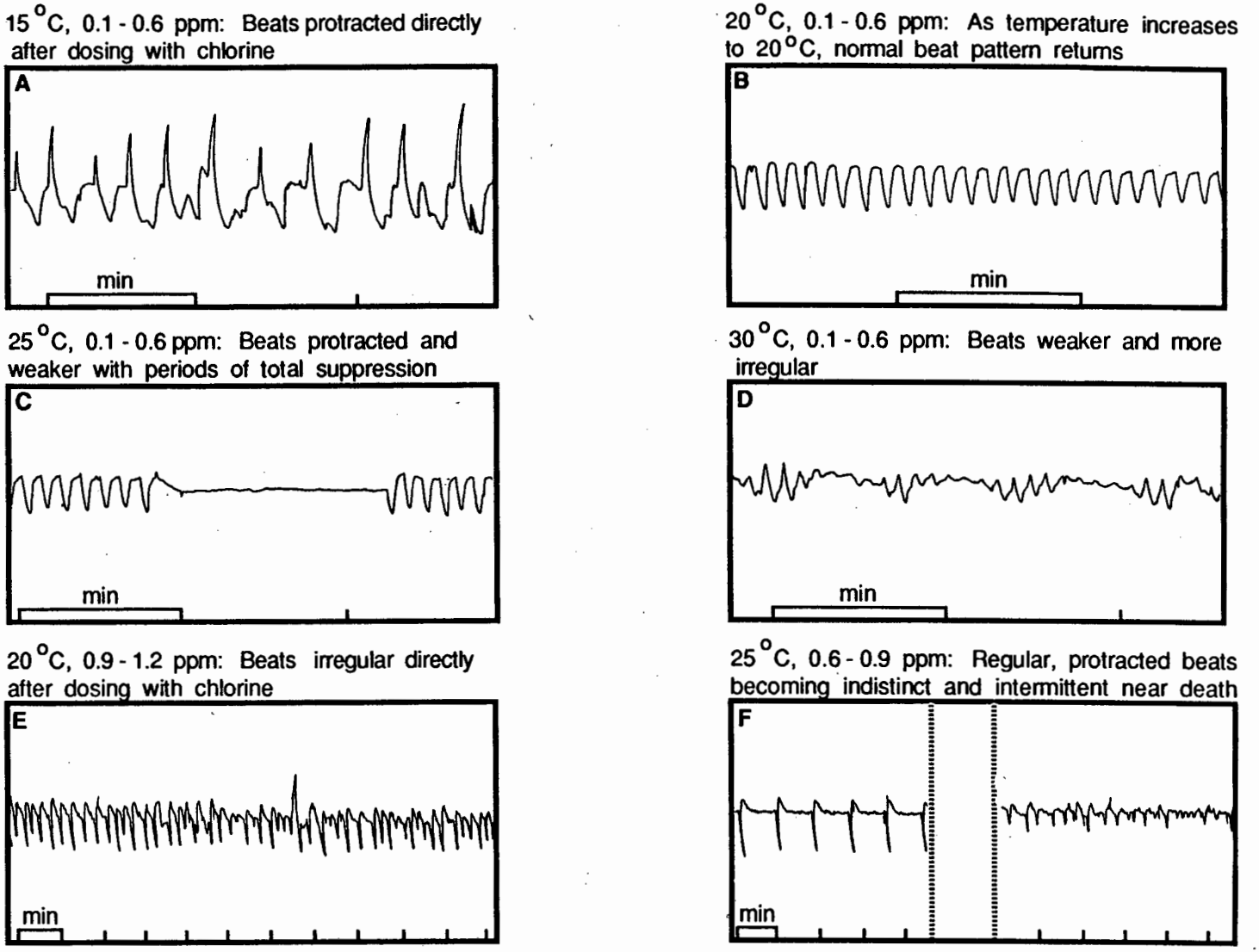

Fig. 2.14. Effect of temperature and chlorine in the range 0.1 - 1.2 ppm on the heart rate of adult individuals using the impedance technique with AC coupling.

DISCUSSION

The effect of temperature on survival

Intertidal distribution of *D. serra* at Ouskip is distinctly size related with the smallest animals at MW, middle sizes between MW and LWS and the largest in the surf zone. Thus the population occupies a number of microhabitats which vary depending on the degree of tidal exposure. This in turn implies different temperature regimes where smallest individuals are exposed to the highest and most fluctuating temperatures. It has been shown in numerous studies that the temperatures to which marine bivalves are exposed in their natural environment are a function of microhabitat plus latitudinal position (Henderson, 1929; Dickie, 1958; Kennedy & Mihursky, 1971 & refs. therein; Ansell *et al.*, 1980a, b; Ansell & McLachlan, 1980). These factors interact with characteristics such as body shape and size, growth and reproductive condition to influence the upper temperature tolerances of a species (Bayne, 1976). Thus size-related differences in *D. serra* (Figs. 2.1b & 2.5b) may be ecologically important even though such differences, after acute exposure at least, proved non-significant according to median lethal times (Fig. 2.2b).

During low water spring tides, temperatures in the sand at depths of 10-15 cm at MW where small *D. serra* are found, are usually warmer than in the surf zone by 1 to 3°C, depending on atmospheric temperature (pers. obs.).

Physiological adjustment to such temperature changes is reflected in LT_{50} values for small *D. serra* being 2 to 3°C higher than for the larger subtidal individuals after 4 to 11 days exposure to near lethal temperatures (Figs. 2.1b & 2.5b). The smaller size would therefore best tolerate heat loading from the power station, not only physiologically, but also by virtue of intermittent exposure to the thermal plume at high tide. It has been shown in *Mytilus edulis* that exposure to a high temperature in 6 hr cycles rather than continuously, improved survival time by 83% (Pearce, 1969).

The greater temperature tolerance of small individuals is further demonstrated by the fact that they burrowed more rapidly than adults at near-lethal temperatures. However, even though the speed of burrowing declined with size, animals of all sizes still retained the ability to burrow. This is of great ecological significance, since burrowing in nature, sometimes to a depth of 30 cm, enables individuals to escape not only predation and dislodgement by rough seas, but also unfavourable temperatures. Indeed, experiments in which mantle cavity and external temperatures were simultaneously monitored in adults, demonstrated that the shell and sand can afford some insulation against temperature changes. Although it is unlikely that the *Donax* population near Koeberg would ever be exposed to lethal temperatures (ca >32°C), sub-lethal effects, such as forced re-emergence from the sand, could be of major importance.

The ability to maintain position is especially important in the immediate vicinity of the outfall where the scouring effect of the effluent water, which can reach a velocity of 80 m^3 sec^{-1}, is most concentrated.

After 8 to 12 days exposure in the laboratory, BT_{50} values for small *D. serra* fell from 29 to 20°C and that of larger ones from 28 to 24°C (Fig. 2.6b). These temperatures are found in the thermal plume at the outfall and long-term exposure is possible during extended periods of onshore north-westerly winds which trap the plume in the surf zone (Rattey & Potgieter, 1987; Chapter 1). If such conditions prevailed, individuals, especially the subtidal adults, would lose their position in the sand and would either be swept ashore to die of desiccation or become prey to sandsharks and seabirds.

D. serra has an extensive geographical distribution in South Africa, ranging from Walvis Bay on the west coast to just north of East London in the east. A comparison between the upper thermal tolerances of the Koeberg population and *Donax* in Algoa Bay on the south coast is particularly interesting. Intertidal distribution in the Algoa Bay population is also size related but is the reverse of the west coast pattern with juveniles in the swash zone at low tide and the adults in the intertidal. A number of reasons, including differences in interspecific competition, food supply and temperature regimes (Donn, 1986), have been proposed for this reversal. On the west coast, sea

temperatures range from 8 to 14°C in summer and from 11 to 17°C in winter (Walker *et al.*, 1984) and food supply is mainly detritus and nearshore phytoplankton which bloom in response to upwelling (see Chapter 4). In Algoa Bay the summer maximum is 26°C with an annual mean maximum of 21 to 22°C (Ansell & McLachlan, 1980) and in winter temperatures drop to 15-17°C (Hanekom, 1975). *D. serra* and another smaller species, *D. sordidus*, which is absent in the west, feed mostly on diatoms sustained within the surf zone in a semi-enclosed ecosystem (McLachlan & Bate, 1984).

LT_{50} data presented by Ansell & McLachlan (1980) for *D. serra* in Algoa Bay are directly comparable with data presented here. After 48 and 72 hrs of acute exposure, large south-coast individuals showed a higher tolerance than small ones (see Table 2.1 later). The burial response also differed in that on exposure to high temperatures, south coast adults burrowed immediately with a BT_{50} after 1 hr of 34.5°C compared to 29.5°C for adults from the west coast which also showed a marked delay in burrowing (Fig. 2.4b). Small individuals from both areas burrowed immediately, but after 1 hr those near Koeberg displayed a BT_{50} of 37°C compared to only 31°C for a similar size from Algoa Bay. These differences suggest that upper temperature tolerances within the two populations are most strongly influenced by different microhabitats (i.e. degree of tidal exposure) rather than size, local temperature or food supply.

A characteristic stability of LT_{50} values after 48 to 72 hrs exposure allows direct comparison of *Donax* species from South Africa with those from European waters (Table 2.1). Such a comparison provides an insight into the interaction between upper thermal tolerances and latitudinal and bathymetric distributions. Ansell *et al.* (1980b) attribute differences between the European species to differences in zonational position on the shore and zoogeographic locality where higher LT_{50}'s correspond to greater tidal exposure and a more southerly distribution. *D. trunculus*, followed by *D. semistriatus*, have the greater thermal tolerances, reflecting the former species distribution in shallower water than the latter and the more southern range of both compared to *D. vittatus* from the North Atlantic. The two South African species compare most closely with *D. semistriatus* and *D. vittatus*; the size-related difference in *D. vittatus* does not reflect different microhabitats but is believed to be an influence of age. In South Africa, *D. sordidus* has the highest thermal tolerance which is indicative of its truly intertidal habitat where it migrates with the tides. However, there is little differences in tolerance between the west and south coast *D. serra* at the same tidal levels and this probably reflects their similar latitudinal positions between 33° and 34°S.

Data for burrowing bivalves of other genera allow for a broader comparison of LT_{50}'s, but only after acute exposure to near-lethal temperatures for 24 hrs. Table 2.2

Table 2.1 Comparison of LT_{50} values meaned over 48 and 72 hrs of exposure when LT_{50}'s had stabilised for *Donax* species from the southern temperate (South Africa), European warm-temperate (Mediterranean) and Mediterranean-boreal (N. Atlantic) [after Ansell & McLachlan, 1980].

Locality	Species	Mean 48-72 hr LT_{50} (°C) size	
		small	large
S. Africa (south coast)	*D. sordidus*	-	29.9
	D. serra	27.1	29.0
S. Africa *(west coast)*	*D. serra*	*29.2*	*28.5*
Mediterranean	*D. trunculus*	*32.9*	*32.6*
	D. semistriatus	-	*30.6*
N. Atlantic	*D. vittatus*	*28.2*	*24.8*

compares bivalves from different shoreline distributions and latitudes in both the northern and southern hemisphere. The high LT_{50}'s among the intertidal group, which compare well with values for South African *Donax*, support the general maxim that molluscs experiencing tidal exposure have the greater thermal tolerance (Henderson, 1929; Southward, 1958; Kennedy & Mihursky, 1971). Indeed, like *D. serra*, the greater tolerance of small size *M. arenaria* and *M. balthica* is directly related to a difference in microhabitat where juveniles are more exposed to the warming of mud flats on the receding tide.

The thermal response of species compared within one study do reflect zoogeographic influences. Between *M. arenaria*, *M. balthica* and *G. gemma*, the latter species, followed by *M. balthica*, have the greatest tolerances coinciding with their wider distribution range on the east coast of the U.S.A. European *T. fabula* and *T. tenuis* from the Mediterranean have higher LT_{50}'s than populations in the North Atlantic, even though *T. tenuis* is subtidal in its southern distribution.

Gradual exposure to increasing temperatures slightly increased the upper temperature tolerances of *D. serra* (Fig. 2.5b). This suggests an ability to adjust upper limits and can be compared to geographically separated *Tellina* species (Table 2.2), as well as to seasonally acclimated *Mya*, *Gemma*, *Macoma*, *Tellina*, and European *Donax*, in which shifts in upper tolerances corresponded to seasonal changes in

Table 2.2 Comparison of LT_{50} values after 24 hrs exposure to near-lethal temperatures for burrowing bivalves acclimated between 15 and 20°C from intertidal and subtidal habitats in the northern and southern hemisphere.

Species	24 hr LT_{50} (°C) size: small	large	Reference
Northern hemisphere subtidal			
Placopecten magillanicus	-	22.5	Dickie (1958)
Tellina fabula (European N. Atlantic)	-	27.0	Ansell *et al.* (1980a)
Tellina fabula (Mediterranean)	-	29.0	Ansell *et al.* (1980a)
Tellina tenuis (Mediterranean)	-	33.5	Ansell *et al.* (1980a)
Donax semistriatus	-	30.0	Ansell *et al.* (1980b)
Donax trunculus	-	33.5	Ansell *et al.* (1980b)
intertidal			
Mya arenaria	31.6	30.5	Kennedy & Mihursky (1971)
Gemma gemma	-	37.2	Kennedy & Mihursky (1971)
Macoma balthica	32.0	31.5	Kennedy & Mihursky (1971)
Modiolus demissus	-	38.4	Waugh & Garside (1971)
Tellina tenuis (European N. Atlantic)	-	31.5	Ansell *et al.* (1980a)
Donax vittatus	-	29.0	Ansell *et al.* (1980b)
Southern hemisphere intertidal			
Donax sordidus	-	33.0	Ansell & McLachlan (1980)
Donax serra	29.0	31.0	Ansell & McLachlan (1980)
Donax serra	32.0	31.0	this study

temperature. Thus it should be borne in mind that LT_{50}'s determined in the laboratory do not represent a finite limit, but rather an average upper tolerance which can assist in understanding, not only an individual species, but also the thermal load that its biotic environment can tolerate. Furthermore, it should be recognised that many factors, including salinity, pO_2, the thermal limits of protein stability (see Chapter 3) and thermal history of an organism (Newell, 1979), interact to influence upper temperature tolerances and each combination of factors can be specific to a species and in some cases to individuals within a species.

The effect of chlorine on survival

In all investigations of the effect of chlorine on *D. serra* concentrations have been measured as free residual chlorine. However, this is a simplistic measure as the chemistry of chlorine in sea water is extremely complex. On the addition of chlorine (NaOCl) to sea water, a variety of halogenated organics are rapidly formed depending on physical and chemical parameters, including but not limited to pH, temperature, sunlight (UV), ammonia concentration, organic load and salinity (Morgan, 1980). Although the effects of these derivatives on marine organisms are as yet not well understood, there is some evidence that they may be more toxic than chlorine itself (Waugh, 1964; Morgan & Carpenter, 1978; Hileman, 1982; Helz & Kosak-Channing, 1984).

Therefore any reference to the toxicity of chlorine to an organism includes the effects of unmeasured derivatives.

The immediate withdrawal of the siphons and foot and valve closure by *D. serra* on dosing with chlorine is a common escape response among burrowing bivalves suddenly exposed to a chemical pollutant, as well as to drastic changes in salinity (Block, 1977; Akberali & Black, 1980; Trueman & Akberali, 1981; Akberali & Davenport, 1982; Trueman, 1983). At low chlorine concentrations (<0.3 ppm) *D. serra* re-emerged and resumed pumping within 3 - 6 hrs of chlorine addition. At levels approaching lethal limits (>0.6 ppm), the valves remained closed for up to 8 days, although full recovery in non-chlorinated sea water only occurred after 6 days. During this time the mantle margins often protruded but since they were always held tightly together, it seems likely they afforded the same protection as sealed valves.

The estuarine bivalve *Scrobicularia plana* can also remain closed for a similar period, 5 to 7 days, in the presence of copper and low salinities (Trueman & Akberali, 1981; Trueman, 1983) and *Mytilus edulis* responded to salinity decline by closing for 4 days (Davenport, 1981). The duration of valve closure must be a critical period because eventually the depletion of energy resources (see Chapter 3) or the accumulation of metabolites would force the animals to open their valves and interact with the environment (Akberali & Black, 1980). Valve closure is thus

effective in isolating tissues from unfavourable conditions provided that these conditions are of a transient or recurrent short-term nature. Such avoidance behaviour is not restricted to bivalves but also occurs in other valved organisms, for example barnacles and cirripede cyprids. It is for this reason that power stations usually chlorinate continuously, since intermittent addition would be ineffective in removing such animals.

At moderate chlorine levels, 0.3 to 0.6 ppm, *D. serra* periodically opened and closed valves and siphons to draw in water, and this action is probably a way of testing external conditions. Hodgson & Fielden (1984) have shown the existence of ciliated sensory receptors on the inner and outer side of both siphons as well as on the lobes of the tentacles and mantle edge of *D. serra* and there are strong indications that these are chemoreceptors. The cruciform muscle complex could be another site of mechano- and chemo-reception as found in other burrowing bivalves (Odiete, 1978; Pichon *et al.*, 1980). Siphonal receptors have also been observed in *Mytilus edulis* (Davenport, 1981) and on the mantle tentacles of the giant scallop, *Placopecten magellanicus* (Moir, 1977) and on *Lima hians* (Owen & McCrae, 1979). The sensitivity of isolated siphonal preparations to low levels of chemical stimuli (Akberali *et al.*, 1981; Hodgson, 1982) is further evidence of the efficiency of chemoreception in bivalves.

The percentage of *D. serra* dying on exposure to a free residual chlorine range of 0.1 to 1.2 ppm was not gradual but rather displayed a stepwise response with the threshold between chronic and acute toxicity at around 0.6 ppm. Above 0.6 ppm median lethal time approximated 10 days but below this concentration, percentage mortality never reached 50% over a period of 14 days. The American oyster *Crassostrea virginica* shows even greater tolerance than *D. serra* with <10% mortality when exposed to chlorine in the range 0.35 to 0.85 ppm for 15 days (Scott & Middaugh, 1978). Such resilience to chlorination was not evident in a model suggested by Mattice & Zittel (1976) which predicted the threshold between acute and chronic toxicity for a heterogeneous array of organisms to be only 0.02 ppm. However this model could be biased towards a low threshold, since a high proportion of fish, which are extremely sensitive to chlorine (Morgan & Carpenter, 1978; Jolly *et al.*, 1978; Hocutt *et al.*, 1980), were included.

Any toxicology study or a study of upper temperature tolerances, should ideally include all stages of the life cycle of the test organism, that is eggs/sperm, larval stages and adults. This was not possible for *D. serra*, since the animal could not be induced to spawn in the laboratory. This is unfortunate as knowledge of the sensitivity of the early life stages is important in assessing the survival ability of a species. The effect of temperature on larvae is possibly demonstrated by a

disruption of the *Donax* breeding cycle during an anomaly in 1982 on the west coast of South Africa (Birkett & Cook, 1987). Inshore temperatures averaged at 5°C higher than normal and this was followed by a failure in the settlement of larvae on the beach. With respect to chlorine, Roberts *et al.* (1975) have shown that very low concentrations over an extended time are generally extremely detrimental to bivalve larvae. For example, 50% of *Crassostrea virginica* and *Mercenaria mercenaria* larvae died after 48 hrs exposure to <0.006 ppm (Waugh, 1964). There is also evidence that chlorine can affect the settling behaviour of larvae by interfering with the detection of pheromones which stimulate settlement (Hillman, 1980).

The effect of temperature and chlorine on heart rate

D. serra showed no acclimation of heart beat frequency from 15 to 25°C. In other studies on bivalves the relationship between temperature and heart beat has always been linear; for example, *Isognomon alatus* (Trueman & Lowe, 1971); *Mya arenaria* (Lowe & Trueman, 1972); *Mytilus edulis* and *M.californianus* (Pickens, 1965; Widdows, 1973); *Crassostrea gigas* (Lowe, 1974) and *Perna perna* (Bayne, 1976). These species, like *D. serra*, displayed Q_{10}'s >2 between 10°C and 25/27°C beyond which beat frequencies generally became erratic, thereby reflecting the critical temperature at which homeostatic mechanisms begin to break down. For *Donax* this coincides with no return to a normal heart beat at 15°C after exposure to 30°C.

As temperature increased, the heart rate of *D. serra* increased immediately, although maximum rates were only reached after a few hours exposure to a raised temperature. Such a rapid response has been noted in other bivalves, as well as isolated heart preparations, and it has been suggested that thermoreceptors, possibly in the mantle tissue, play an important role in the respect of an immediate response of heart muscle to temperature change (Trueman & Lowe, 1971; Lowe, 1974). Since there is an abundance of sensory cells on the siphons and mantle edge of *D. serra*, even though most appear adapted to detect chemical or mechanical changes in the external medium (Hodgson & Fieldin, 1984), it seems reasonable to assume that at least some of these cells function as thermoreceptors with a possible neural connection to the heart.

The addition of chlorine at all concentrations between 0.1 and 1.2 ppm to sea water at 15°C had the same effect on *Donax* as a temperature change in that the heart responded immediately, possibly via the chemoreceptors mentioned above. However, beat frequency did not increase but rather drastically declined as *Donax* rapidly withdrew the siphons and foot and closed the valves tightly. As mentioned earlier, this is a common stress response among bivalves to a pollutant, a salinity drop or aerial exposure and in all these instances valve closure is accompanied by a decline in heart rate (Trueman, 1967; Coleman & Trueman, 1971; Earll, 1975; Akberali & Black, 1980; Trueman & Akberali, 1981).

Valve closure leads to a drastic drop in pO_2 and an increase in pCO_2 levels of the mantle cavity water in *M. edulis* (Bayne, 1971) and *S. plana* (Akberali & Trueman, 1979) and it is this drop in oxygen tension, rather than any mechanical effect of closed valves, which is believed to slow down beat frequency (Bayne, 1976).

At 15°C there was a slight recovery in heart beat frequency of *D. serra* when exposed to chlorine <0.3 ppm for 24 hrs and this was paralleled by increasing shell gape and pedal and siphonal extension. It therefore seems likely from this postural behaviour that with extended exposure to low concentrations, the animal would come to tolerate chlorine and perhaps beat frequency would improve. This supposition is supported by the low percentage mortality of *Donax* exposed to 0.1 - 0.3 ppm chlorine at 15°C for up to 2 months in experiments described in Chapter 3. However, above 0.3 ppm heart beats remained suppressed from a normal rate of 13 to about 5 beats min^{-1} for the 24 hrs of the experiment. Since *Donax* is able to keep its valves closed for 7 to 8 days as protection against chlorine, it is probable that during this time heart beat would remain suppressed and anaerobic respiration, which is well documented among bivalves (reviewed by de Zwaan, 1977), would sustain basal metabolism. Indeed, preliminary investigations by A. N. Hodgson of Rhodes University (Grahamstown) suggest that *D. serra* does respire anaerobically when the valves are closed. The accumulated

end-products of anaerobiosis could be buffered by calcium mobilised from the calcareous shell as occurs in *S. plana* (Akberali, 1980; Akberali *et al.*, 1977).

When the temperature was raised to 20°C and then 25°C in the chlorine range 0.1 to 0.6 ppm, valve adductions increased and heart beat gradually rose, although the frequencies reached in the absence of chlorine were never attained. The suppressive nature of chlorine is even more dramatically demonstrated above 0.6 ppm when the effect of temperature was completely over-shadowed. Although the valve gape did increase with temperature at these higher chlorine concentrations, this was a passive action rather than controlled valve movement. The adductor muscles became too weak to hold the valves closed, thus making it possible for the hinge ligament to pull them apart.

Recovery at 15°C without chlorine was rapid, but only for individuals exposed for 24 hrs to 0.1-1.2 ppm at 15°C and <0.6 ppm at 20 and 25°C. In fresh sea water sharp valve adductions were accompanied by an overshoot in heart rate which can be associated with an oxygen debt incurred during valve closure. In response to declining oxygen tensions, *D. serra* has been shown to be an oxyconformer incurring an oxygen debt (Van Wijk *et al.*, 1989). Other bivalves have shown a similar recovery response, not only after exposure to a pollutant (Akberali & Black, 1980; Trueman & Akberali, 1981), but also on re-immersion after aerial exposure (Trueman, 1967; Coleman & Trueman, 1971). Individuals which

displayed passive gaping never recovered in non-chlorinated water and this seems, at least partially, a result of total paralysis of all muscles especially in the foot, siphons, adductors and heart. Thus chlorine >0.6 ppm in combination with raised temperatures causes sublethal effects within 24 hrs from which *D. serra* did not recover.

CONCLUSIONS

1. The upper temperature tolerances estimated for *D. serra* in terms of **median** mortality indicate that the population near the outfall of the power station would experience no adverse effects from the thermal plume. However, Schubel *et al.* (1978) have critised the use of only 50% lethal limits and suggest that a family of mortality curves be used ranging from 10% to 90% in intervals of 10%. Such an approach would cover the eventuality of unmeasured sublethal effects and provide a broader estimate of thermal tolerances. In this study estimates of burrowing success provided a median sublethal measure which showed that *Donax* are forced to the surface by temperatures which are not necessarily lethal. Such a response must be considered when assessing effects of the warmed effluent at Koeberg since the loss of anchorage in the sand means eventual death, either by loss of thermal insulation provided by burial or predation or by desiccation once stranded on the beach.

2. In the laboratory *D. serra* is able to protect itself against a wide range of chlorine levels as long as exposure does not exceed 6 days. During this period the animal remains buried and effectively isolated from the environment by closing the valves, but at the same time still retaining an ability to detect and respond to external changes via chemoreceptors on the mantle margin. Nevertheless, on the wave-swept beach at Koeberg, such a response could result in dislodgement from the sand if the animal is not buried deeply with lethal consequences as described above. However, this danger is far less at chlorine concentrations <0.3 ppm, since *Donax* is able to resume burrowing and pumping within 3 - 6 hrs after the initial closure response.

3. *D. serra* is far more efficient in avoiding and protecting itself against temperature increases and chlorination than pelagic organisms since the valves provide a means of isolating internal organs and the sand affords insulation. Pelagic animals, especially fish, cannot isolate vital organs like the gills and their only protection is to move away from unfavourable conditions. This difference in avoidance strategies is reflected by a much greater sensitivity on the part of fish and zooplankton to chlorine (Schubel *et al.*, 1978; Hocutt *et al.*, 1980).

4. The monitoring of heart rate in *D. serra* has proved a convenient method to ascertain responses to thermal and chemical changes in the environment, but only at the level of extreme activities such as burrowing or valve closure.

More subtle, long-term effects cannot be ascertained as no information is gained on the ways in which metabolic energy output is being apportioned and expended. It is from this point that the remainder of this thesis is developed to examine sublethal effects of temperature (15 - 25°C) and chlorine (0.1 - 0.3 ppm) that could alter the physiological fitness of the individual and the population near the thermal discharge.

CHAPTER THREE

BIOCHEMICAL COMPOSITION AND EFFECTS OF LABORATORY CONFINEMENT, TEMPERATURE AND CHLORINE

INTRODUCTION

Most studies on the biochemical reserves of marine bivalves have concentrated on changes in composition in association with the seasonal cycle of gametogenesis, especially in temperate and boreal species (Gabbott, 1975, 1976, 1983; Bayne, 1976; Sastry, 1979; Hawkins *et al.*, 1985). Generally it has been found that during periods of surplus food availability, these bivalves synthesise and store lipid, protein and carbohydrate substrates. Subsequent utilisation has been attributed to conversion of these reserves into lipids within the developing gametes followed by spawning (Gabbott, 1975; Bayne, 1976; Zandee *et al.*, 1980). Reserve depletion has also been related to overwintering stress when metabolic demands still have to be met in the face of low temperatures and food scarcity (Gabbott & Bayne, 1973; Dare & Edwards, 1975; Zandee *et al.*, 1980).

Studies concerned with reserve utilisation as an index of stress factors other than natural ones, are far less common. In this regard, prolonged laboratory confinement, even when simulating natural conditions, has been identified as a stress factor in *Mytilus edulis* (Bayne & Thompson, 1970) and *Donax vittatus* (Ansell & Sivadas, 1973). In these species and in *Tapes japonica* (Mann & Glomb, 1978) any deviation from an ambient temperature regime in laboratory experiments represented severe stress. This was characterised by a marked decline in reserves accompanied by weight loss and gonad recession.

Biochemical composition is thus closely coupled to natural and atypical changes in the environment and as such provides a useful indicator of a bivalve's condition. Against this background, changes in the biochemical condition of *D. serra* were investigated in relation to the temperature regime and chlorine concentration corresponding to that of effluent sea water from Koeberg Nuclear Power Station. Measures of condition involved not only biochemical changes, but also gametogenic development and weight loss. Comparison of biochemical composition of freshly collected animals with those retained in the laboratory under simulated ambient conditions, served to demonstrate effects of confinement.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

D. serra was maintained in the laboratory for a period of 90 days (April - June, 1986) at 15, 20 and 25°C either without or with chlorine in the range 0.1 - 0.3 ppm. This sublethal dose of chlorine was administered as described in Chapter 2. Animals were sacrificed for biochemical analysis at the beginning of experiments and weekly within the first two weeks but only every two weeks thereafter. Treatment at 15°C with no chlorine represented a laboratory control while animals sampled from Ouskip at two-weekly intervals during

experiments acted as natural controls. A temperature of 15°C closely approximates the mean temperature near Ouskip during the months April to June (Birkett & Cook, 1987).

Three size groups were recognised based on shell width as in Chapter 2 and corresponding to dry weights of >1.0 g (large), >0.1 <1.0 g (medium) and <0.1 g (small). For each treatment, animals were kept buried in sand in 60 litres of circulating and well-aerated sea water. Sand was replaced once a week and freshly-sampled surf water was provided twice-weekly but sometimes more often, depending on the daily measure of ammonia-N concentration. Salinity was adjusted by adding double-distilled water. Each day throughout the experiments bivalves were fed a mixture of cultured-algal species plus pre-frozen seafoam which had been collected after stranding on Ouskip beach.

BIOCHEMICAL ANALYSIS

Tissue preparation

For each treatment, two bivalves from each size group were removed from experimental tanks and collected from the field at the above specified times. Medium and small individuals were immediately frozen. Gonad material and the mantle were excised from large animals before freezing.

It was not practical to sacrifice more than 2 individuals per size group. Constraints on numbers were imposed by limits on tank space, overcrowding, natural mortality and mortality due to chlorine tolerance testing.

Since only a few animals were available, it was not possible to undertake statistical analysis of differences in biochemical composition in relation to temperature and chlorine.

All sacrificed animals (minus shells) plus gonad and mantle tissue were freeze-dried for 5 days before homogenising into fine powder. Tissues were subjected to protein, free reducing sugar (FRS) and total carbohydrate (TC) analyses based on an adaptation of the micro-analytical scheme of Holland & Gabbott (1971). Ash content of all tissue was determined by combustion of sub-samples at 450°C for 15 hrs. Biochemical composition and ash content were expressed as percentages of body, gonad or mantle dry weight.

Percentage lipid content was estimated by difference assuming that the gravimetric sum of protein, FRS, TC, ash and lipid equalled the animal dry weight. Unfortunately estimates of percentage lipid were inconsistent (range of 0 - 40%) and showed no trend that could be associated with temperature and chlorine. These results were therefore discarded. Researchers have often found that even when all three major biochemical constituents are accounted for, summation seldom balances (Ansell, 1974c; Ansell *et al.*, 1980c; Epp *et al.*, 1988). Percentages of protein, FRS and TC in this study therefore indicate absolute rather than relative amounts per unit dry weight. By contrast, ash content is a relative measure, always being the reciprocal

of changes in the percentage organics (i.e. the biochemical constituents) of body tissue.

For biochemical analysis, 30 mg of tissue from each individual (i.e. whole body tissue, gonad or mantle) was thoroughly homogenised with 5 ml of double-distilled water. From this homogenate, sub-samples were extracted for analysis of protein, free reducing sugars and total carbohydrate as outlined in the following protocol:

Protein analysis

(modified from Holland & Gabbott, 1971)

to 50 *u*l of tissue homogenate add 50 *u*l 5N·NaOH

heat 30 mins at 56°C after sealing test tube

centrifuge at 800 *g* for 10 mins & collect supernatant

divide supernatant into 3 X 20 *u*l samples

add 30 *u*l distilled water to each 20 *u*l sample

to each of these samples plus standards & blanks (see below), add 1 ml Reagent A (see below)

stand 10 mins at room temperature

add 100 *u*l Reagent B (see below)

mix immediately

stand 30 mins at room temperature

add 1 ml distilled water

read on spectrophotometer at 750 *n*m

REAGENTS:

A - mix 50 ml of 2% Na_2CO_3 in 0.1N·NaOH with 1 ml of 0.5% $CuSO_4 \cdot 5\ H_2O$ in 1% sodium citrate just before use

B - dilute BDH Folin-Ciocalteau reagent with distilled water in the ratio 1 : 1.3 respectively

STANDARDS:

3 X 50 *ul* of 5, 10, 25 & 50 *ug* Bovine Serum Albumin in 50 *ul* 1 N·NaOH

BLANKS:

3 X 50 *ul* 1 N·NaOH

Free reducing sugar and total carbohydrate

(modified from Holland & Gabbott, 1971)

to 200 *ul* of tissue homogenate add 100 *ul* cold 15% TCA

shake in a water bath for 5 mins

stand 10 mins at 4°C

centrifuge at 800 *g* for 10 mins & collect supernatant

wash precipitate with 200 *ul* 5% TCA

centrifuge at 800 *g* for 10 mins & add to previous supernatant

divide combined supernatants into 4 X 100 *ul* samples

free reducing sugars

to 2 X 100 *ul* samples add

20 *ul* 6 N·HCl

20 *ul* 6 N·NaOH

60 *ul* 5% TCA

mix thoroughly

total carbohydrate

to 2 X 100 *ul* samples add

20 *ul* 6 N·HCl

hydrolise at 95°C for 2 hrs

add 20 *ul* 6 N·NaOH & 60 *ul* 5% TCA and mix

To these 4 samples plus standards and blanks (see below) add

500 ul 0.625% NaOH

100 ul 0.2% potassium ferricyanide

200 ul of (500 ul 16% Na_2CO_3 + 30 ul 5% KCN made up to 10 ml with distilled water)

mix well and heat at 95°C for 10 mins

cool & add 500 ul distilled water

mix and then centrifuge for 5 mins at 800 *g*

read supernatant on spectrophotometer at 420 *nm*

STANDARDS:

3 X 200 ul of 2.5, 5, 10 & 15 ug glucose in 200 ul 5% TCA

BLANKS:

3 x 200 ul 5% TCA

GAMETOGENIC STAGES

Before freezing, the gametogenic stage of adults was identified from fresh gonad smears. Fractions of gonad were placed on a glass-slide with a drop of Rose Bengal and glycerine. Although not as comprehensive as histological preparations, these smears did enable the reliable identification of 4 gametogenic stages:

(0) - CYTOLYSED STATE in which gonads appear completely degenerated; there is very little reproductive tissue, alveoli and germ cells cannot be clearly discerned and gonads generally are brick-red in colour.

(1) - INACTIVE OR SPAWNED STATE - these two conditions are grouped by having large, relatively empty alveoli either

ruptured or with some follicles or/and a thin layer of germ cells indicative of either inactivity or recent spawning.

(2) - DEVELOPING STATE - early active condition in which the alveoli are filled with germ cells in various stages of development.

(3) - RIPE STATE - late active and pre-spawning condition in which alveoli are filled with fully mature gametes and the amount of gonad is vast, extending deep into the foot.

CONDITION INDICES

As an index of weight loss during the 90-day period, dry tissue weight was compared with shell weight and shell volume assuming that these two latter measures would not change significantly during experiments. Field animals served as natural controls. After freeze-drying and prior to homogenising, whole body tissue and shell valves were weighed separately. Since the two valves are symmetrical, the volume of only one was measured by filling the valve cavity with water, recording the volume and doubling it.

RESULTS

BIOCHEMICAL COMPOSITION

Whole body tissue

Size-related changes in protein, FRS, TC and ash content of whole tissue (as % dry weight) over the 90-day period are presented in Figs. 3.1 - 3.4. The biochemical composition of field animals is compared to those exposed to 15, 20 and 25°C without and with chlorine (0.1 - 0.3 ppm).

Protein (Fig. 3.1)

In field animals, protein comprised between 60 - 80% of tissue dry weight. The highest percentage was recorded in large bivalves, most probably because of their proportionally larger muscular foot and well-developed adductor muscles. Protein levels in control laboratory animals (15°C, no chlorine) were similar to field values in the first 20 - 30 days of experiments. Thereafter, however, control laboratory animals lost about 10% more protein than field animals. This indicated that long-term laboratory confinement (>30 days) was slightly detrimental to protein conservation.

The effects of elevated temperature were, however, clearly demonstrated on referral to controls. At both 20 and 25°C, protein content decreased rapidly in the first 20 days by about 30 - 40%. In the latter half of the experiment, protein losses were minimal. Nevertheless,

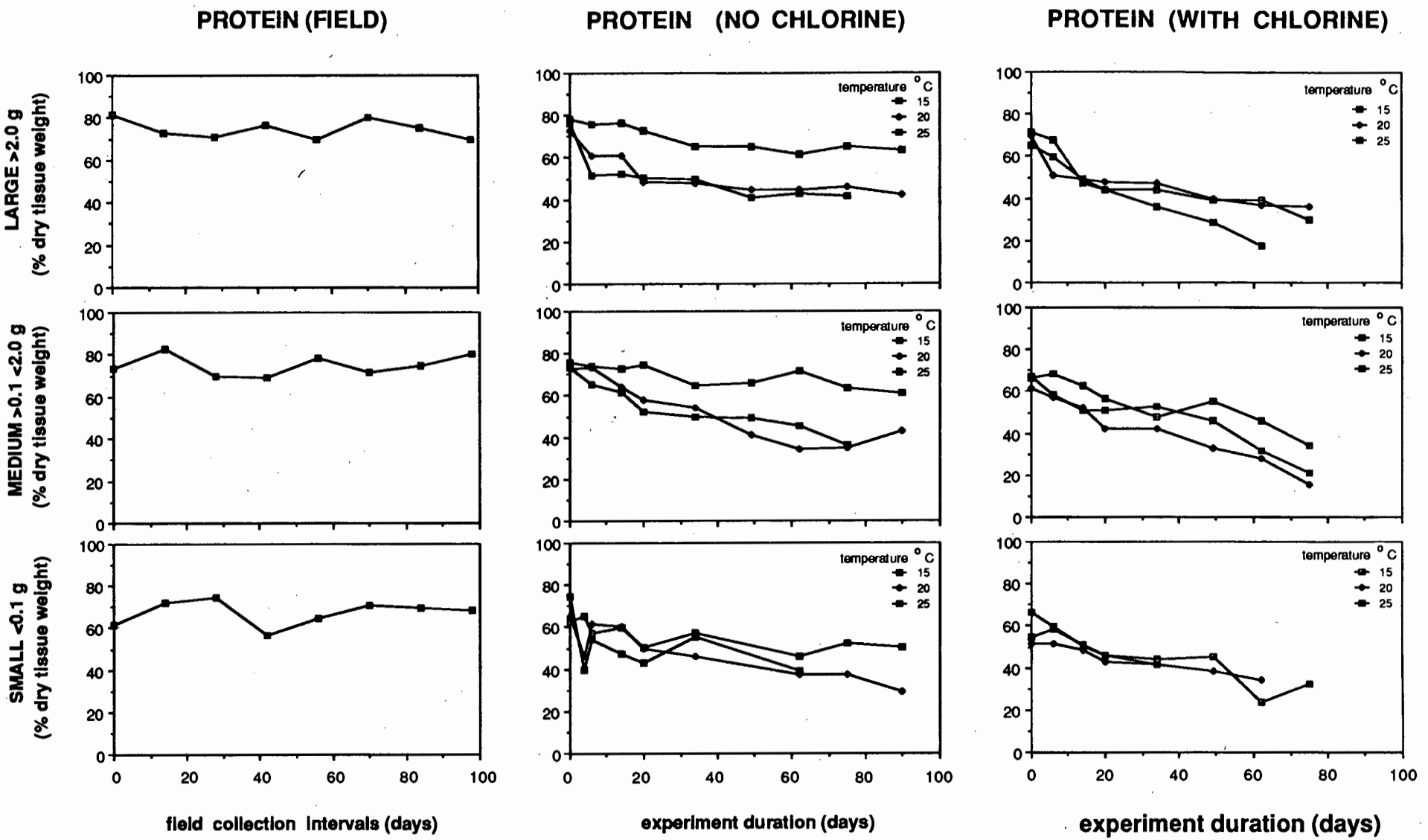

Fig. 3.1. PROTEIN content as percentage of whole body tissue (g dry) in three size groups of *D. serra* . Animals freshly sampled from the field are compared with those exposed to temperatures from 15 - 25 °C without and with chlorine (0.1 - 0.3 ppm) for a period of 90 days. Each data point is the mean of 2 values.

small animals died after 60 days while medium and large individuals died after 75 days exposure to 25°C. At this point protein reserves were approximately half the initial amount.

The addition of chlorine (0.1 - 0.3 ppm) to control animals (15°C) caused a more rapid loss in protein content over 75 days (30 - 40%) than occurred at 15°C only. The synergistic effect of elevated temperature (20 and 25°C) and chlorine resulted in a more pronounced loss of protein (up to 50%) than the effect of chlorine additions at 15°C. The combined effect of chlorine and elevated temperature was slightly greater (10 - 15%) than elevated temperature alone. With this treatment animals had died by day 75 and at this stage protein content had fallen to as low as 20%. Clearly chlorine had a far greater detrimental effect on protein reserves than elevated temperature.

Free Reducing Sugars (Fig. 3.2)

Natural FRS levels were between 5 and 8% of total dry body weight. Lowest values were found in juveniles and highest in medium and large individuals. Adults displayed marked fluctuations in sugar reserves, perhaps reflecting variations in gametogenic condition.

There was only a slight indication of sugar loss due to confinement at 15°C. At 20 and 25°C, FRS reserves declined in an irregular but consistent manner so that their percentage contribution to dry tissue weight had dropped

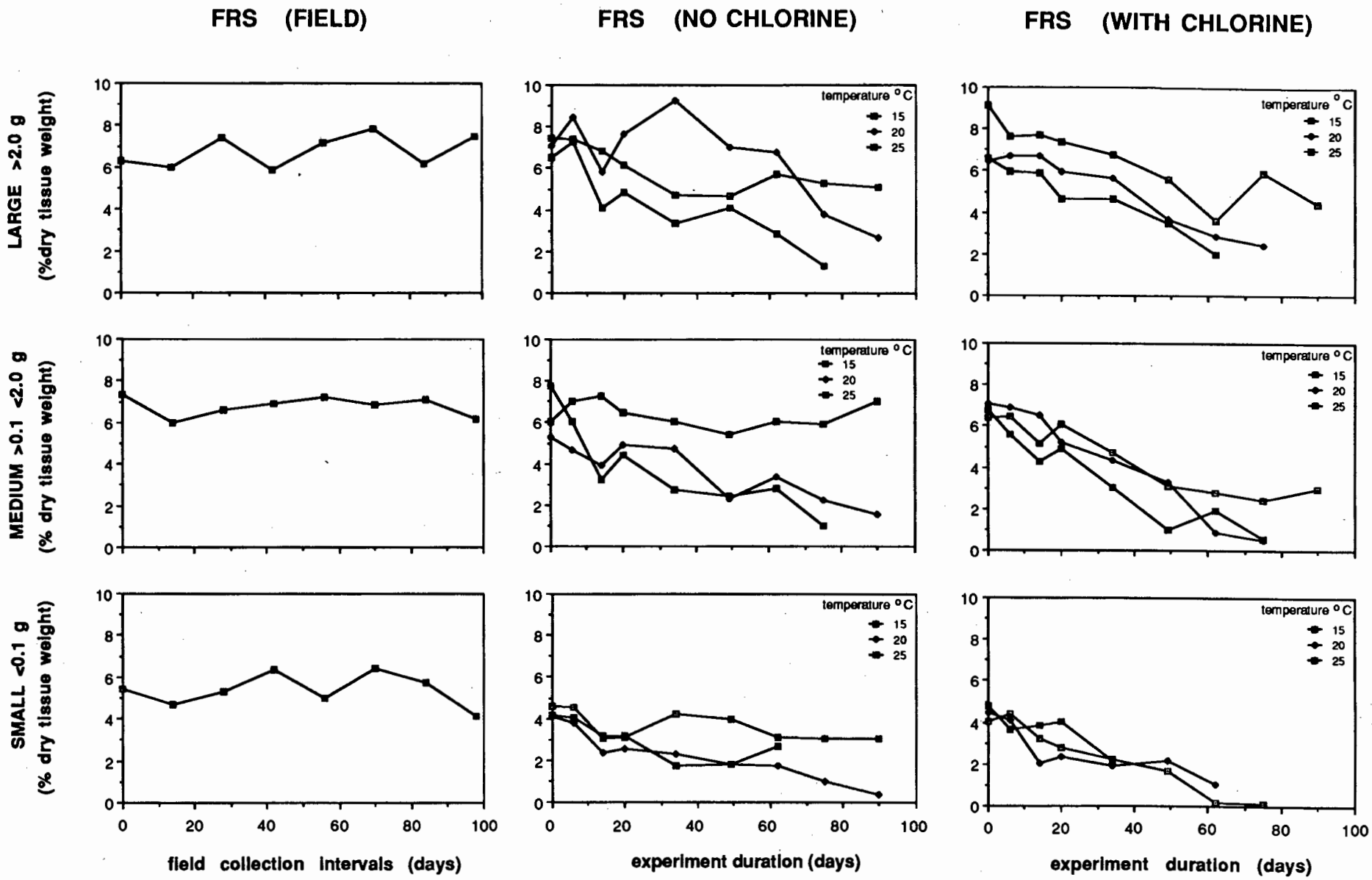

Fig. 3.2. FREE REDUCING SUGAR content as percentage of whole body tissue (g dry) in three size groups of *D. serra*. Animals freshly sampled from the field are compared with those exposed to temperatures from 15 - 25 °C without and with chlorine (0.1 - 0.3 ppm) for a period of 90 days. Each data point is the mean of 2 values.

from about 8% to 2% in >0.1 g *Donax* and from 5% to 1% in juveniles by the end of the experiments.

Addition of chlorine further decreased reserves relative to treatment without chlorine. In medium and small sizes, the rate of this decline was similar at all three temperatures. However, at 15°C adults showed some resilience to chlorine since sugar reserves were maintained at levels only fractionally below those recorded at 15°C without chlorine.

Total carbohydrate (Fig. 3.3)

Total carbohydrate, that is mono- plus poly-saccharides, comprised between 7% and 9% of body tissue in *Donax* >0.1 g and a little less (6 - 7%) in small ones collected from the field. These values were only between 1 and 3% greater than FRS composition (Fig. 3.2) indicating that reducing sugars were a significant subfraction of total body carbohydrate.

Total carbohydrate content differed little between field and laboratory controls, although in the latter, percentage composition was more variable with time. This variability is attributable to the fluctuations in FRS levels (Fig. 3.3).

The effect of exposure to 20 and 25°C closely paralleled the irregular decline observed in FRS levels at these temperatures. (Fig. 3.2). Depletion in carbohydrate reserves was less at 20°C than 25°C in adult bivalves but such a temperature-related difference was not apparent in smaller size groups.

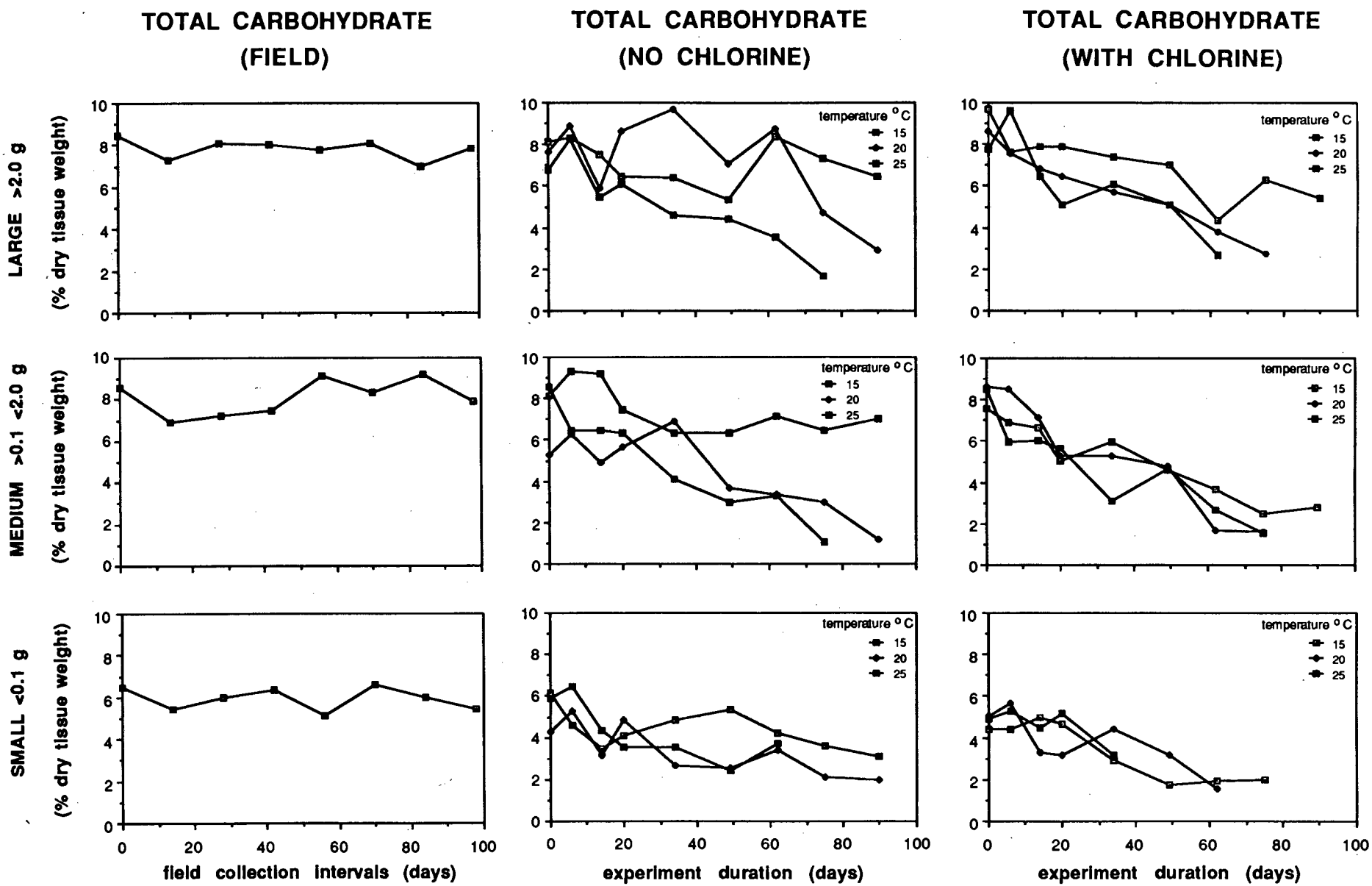

Fig. 3.3. TOTAL CARBOHYDRATE content as percentage of whole body tissue (g dry) in three size groups of *D. serra*. Animals freshly sampled from the field are compared with those exposed to temperatures from 15 - 25 °C without and with chlorine (0.1 - 0.3 ppm) for a period of 90 days. Each data point is the mean of 2 values.

The presence of chlorine further depleted total carbohydrate content in much the same manner as observed in FRS decline. Adults again showed some tolerance of chlorine at 15°C as TC reserves were conserved.

Ash content (Fig. 3.4)

Ash content in field animals was size-related, being between 12 - 14% of adult dry tissue weight, 10 - 13% in medium sizes and 20 - 24% in small ones. As may be expected from the stability of biochemical constituents (= organic fraction), the percentage ash content changed little at 15°C in the laboratory.

High temperatures and chlorine exposure resulted in the elevation of the proportion of ash in body tissue. The extent of these increases (10 - 20%) is simply a function of the decline in protein, FRS and TC (Figs. 3.1 - 3.3).

Gonad and Mantle Tissues

Biochemical composition and ash content of these tissues excised from adult *D. serra* are presented in Figs. 3.5 - 3.8 in relation to natural conditions, temperature, chlorine and experiment duration.

Protein (Fig. 3.5)

Protein reserves in the gonad of field animals were low (30 - 50% of gonad dry weight) relative to that found in the mantle (55 - 75% of mantle dry weight). Confinement in the laboratory at 15°C did not alter protein percentage other than inducing greater variability in data. At 20 and 25°C, gonad protein loss was gradual with time (a drop from about

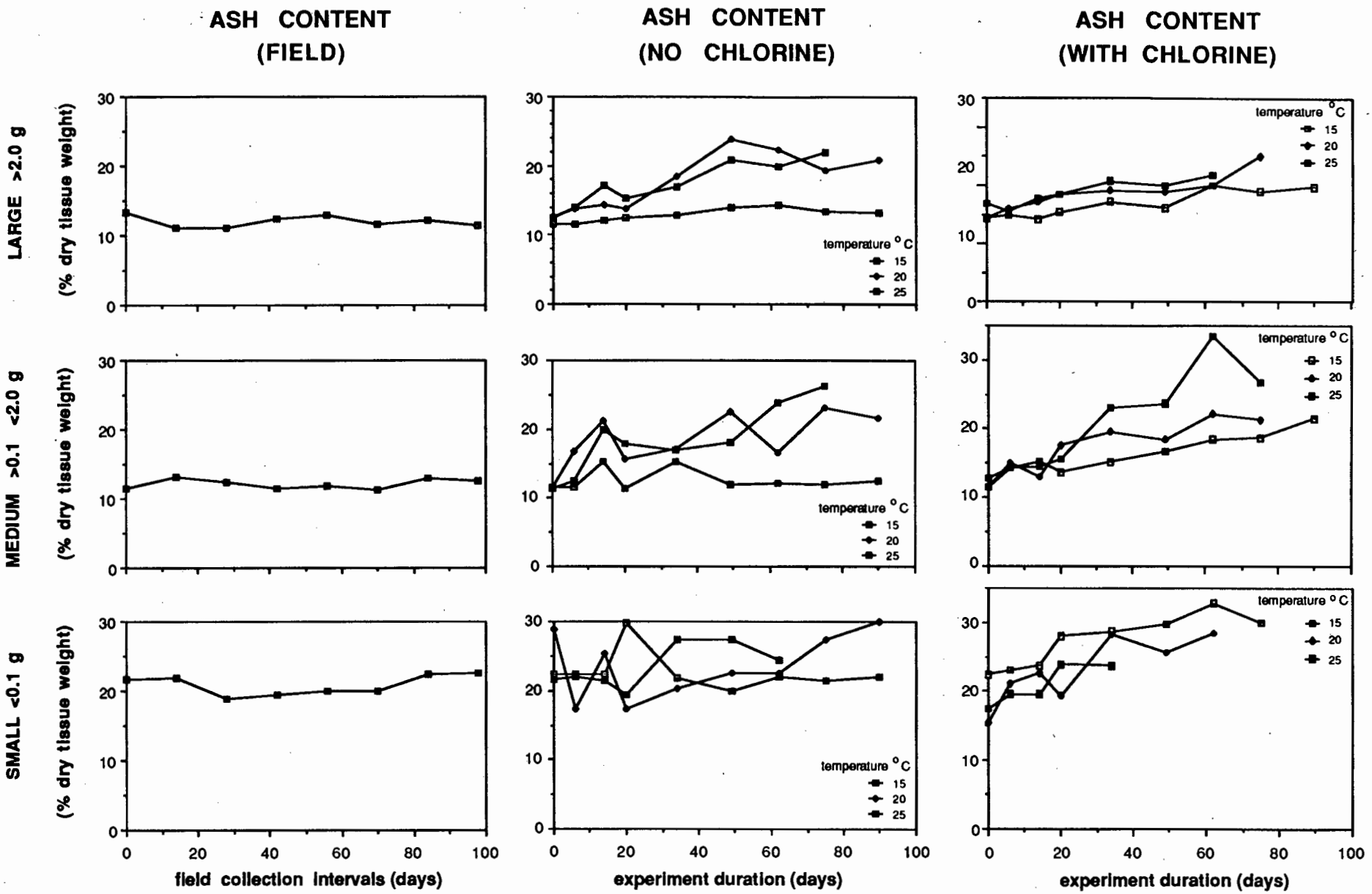

Fig. 3.4. ASH content as percentage of whole body tissue (g dry) in three size groups of *D. serra* . Animals freshly sampled from the field are compared with those exposed to temperatures of 15, 20 & 25 ° C without and with chlorine (0.1 - 0.3 ppm) for a period of 90 days. Each data point is the mean of 2 values.

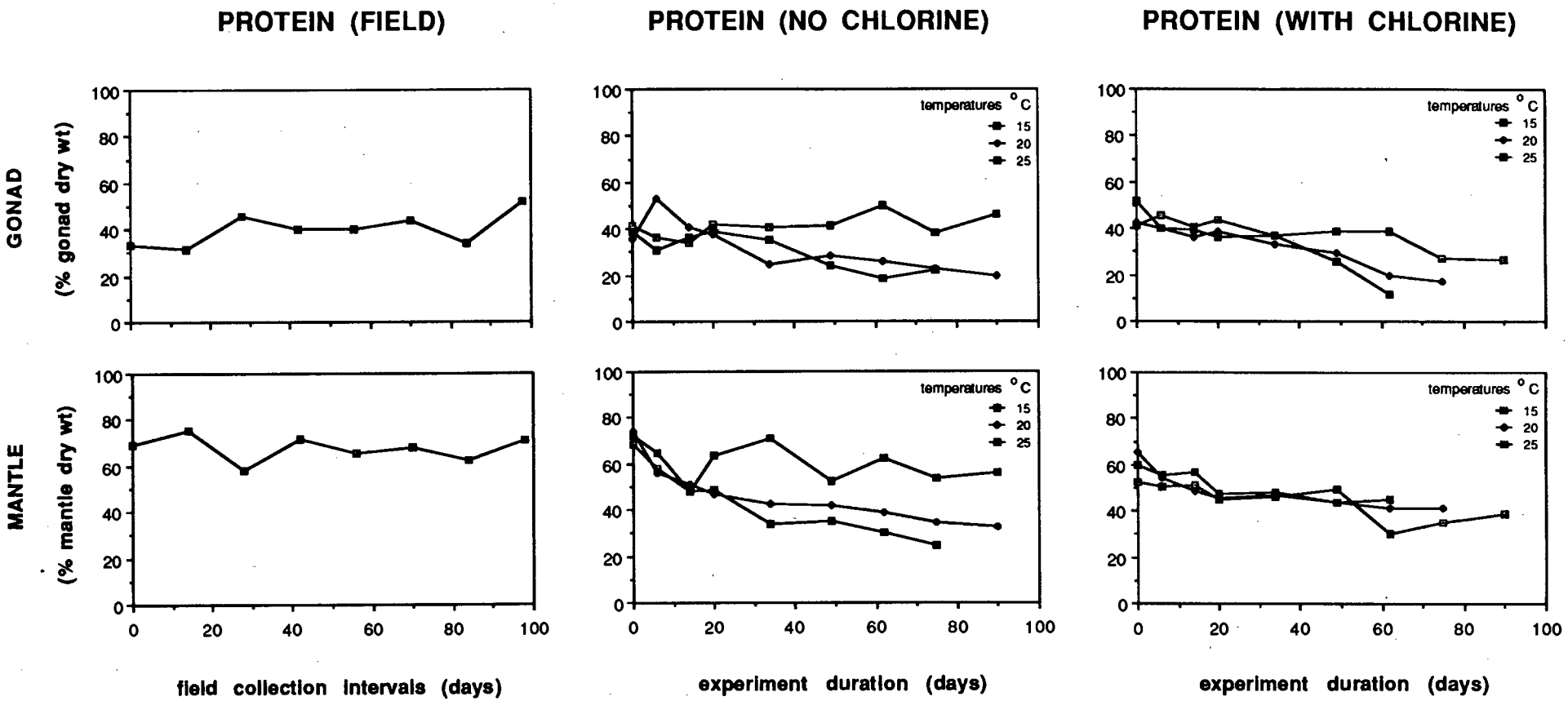

Fig. 3.5. PROTEIN content as percentage of dry weight of gonad and mantle in adult *D. serra* . Animals freshly sampled from the field are compared with those exposed to temperatures of 15, 20 & 25 °C without and with chlorine (0.1 - 0.3 ppm) for a period of 90 days. Each data point is the mean of 2 values.

40 to 25%). On the other hand, protein stores in the mantle were rapidly utilised (85% down to 55%) in the first 20 days at these temperatures. Thereafter depletion continued, but at a much slower rate.

Exposure to chlorine at 15°C resulted in a small protein loss in the reproductive tissues whereas reserves in the mantle were quickly diminished. Chlorine addition at higher temperatures marginally increased gonad protein losses relative to non-chlorine treatment. In the mantle, however, protein losses at elevated temperatures exceeded those under the combined effects of chlorine and raised temperature.

Free Reducing Sugars (Fig. 3.6)

Reducing sugars comprised 8 - 9% of gonad tissue but only 3 - 5% of the mantle in freshly-collected *Donax*. At 15°C in the laboratory, reserves in these tissues closely matched that in nature but displayed more variability with time.

With an increase in temperature, gonad FRS content rapidly fell from around 8 to 3%, the rate of decline being more pronounced at 25°C. The small FRS reserve in the mantle was rapidly utilised within the first 10 days exposure to 20 and 25°C, but thereafter percentage composition fluctuated irregularly.

At 20 and 25°C, chlorine treatment caused no further decline in FRS levels in either tissue fraction. However, at 15°C reserves were markedly depleted relative to non-chlorinated experiments. This decline was rapid in the

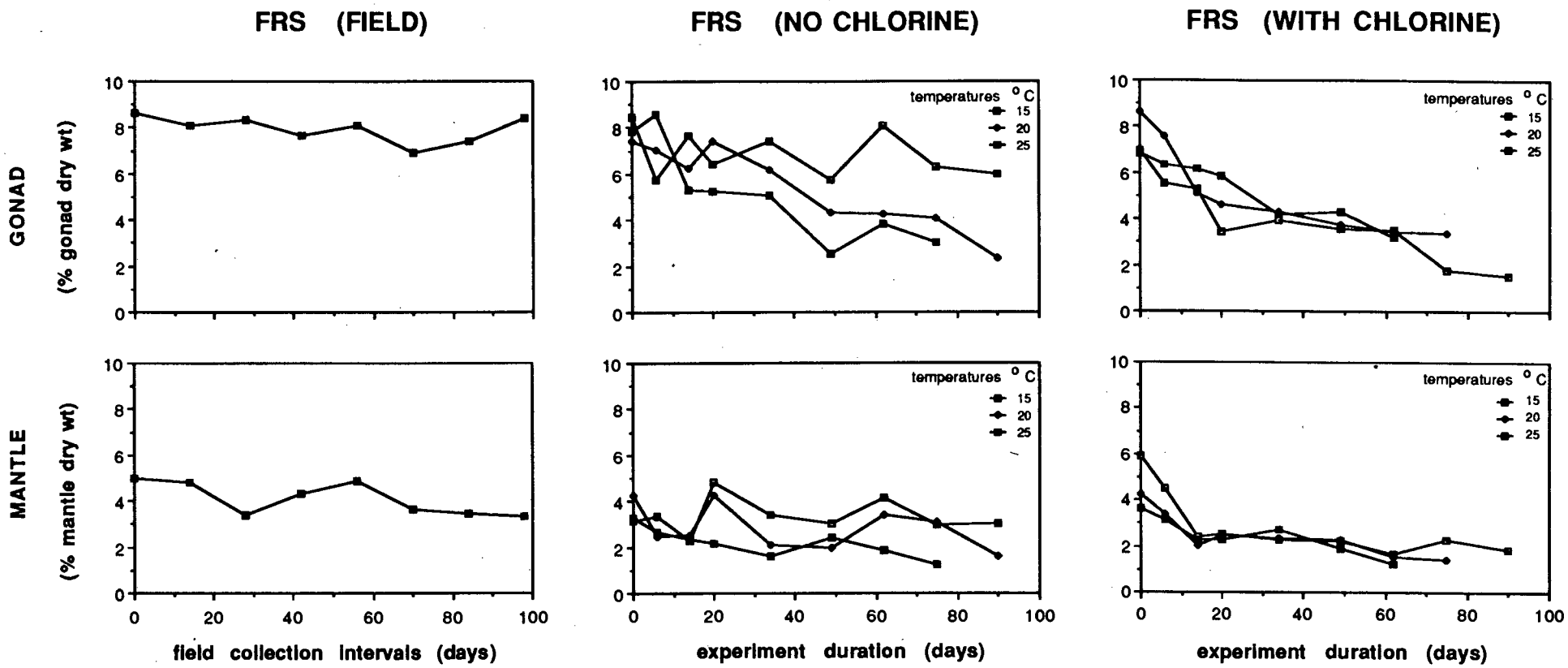

Fig. 3.6. FREE REDUCING SUGAR content as percentage of dry weight of gonad and mantle in adult *D. serra* . Animals freshly sampled from the field are compared with those exposed to temperatures of 15, 20 & 25 °C without and with chlorine (0.1 - 0.3 ppm) for a period of 90 days. Each data point is the mean of 2 values.

first 10 to 20 days but later slowed down to reach a steady composition of 2 to 3%.

Total carbohydrates (Fig. 3.7)

As in whole body tissue, total carbohydrate content in the gonad and mantle of field and experimental animals was only slightly more than FRS levels. Even in these tissue subfractions it seems that FRS dominates total carbohydrate composition. Consequently, any changes in total carbohydrates associated with elevated temperature and chlorine follow the same pattern as observed for FRS (Fig. 3.6).

Ash content (Fig. 3.8)

Reproductive tissue from field animals had a lower inorganic content (5 - 10%) than in the mantle (22 - 25%). Reciprocally, therefore, gonads were much higher in organics. Under laboratory simulated ambient conditions (15°C), gonad condition, as indicated by the organic to inorganic ratio, deteriorated only slightly after 50 days confinement. By contrast, the ash content of mantle tissue was essentially unaltered in the laboratory.

In both tissue fractions, temperature increase with or without chlorine caused a loss of protein, TC and FRS reserves as described previously. These losses are reflected in the increased percentage ash content of approximately 5 - 10% in the tissues.

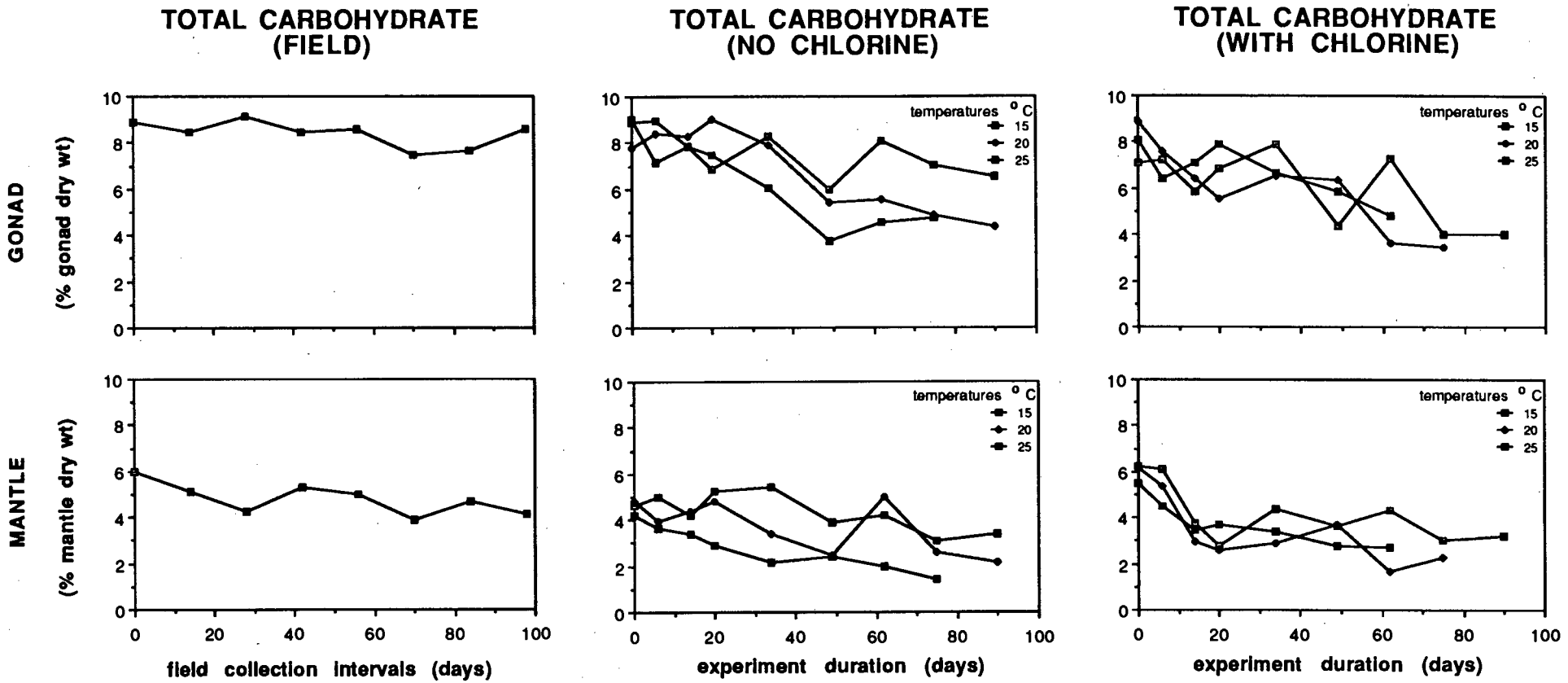

Fig. 3.7. TOTAL CARBOHYDRATE content as percentage of dry weight of gonad and mantle in adult *D. serra* . Animals freshly sampled from the field are compared with those exposed to temperatures of 15, 20 & 25 ° C without and with chlorine (0.1 - 0.3 ppm) for a period of 90 days. Each data point is the mean of 2 values.

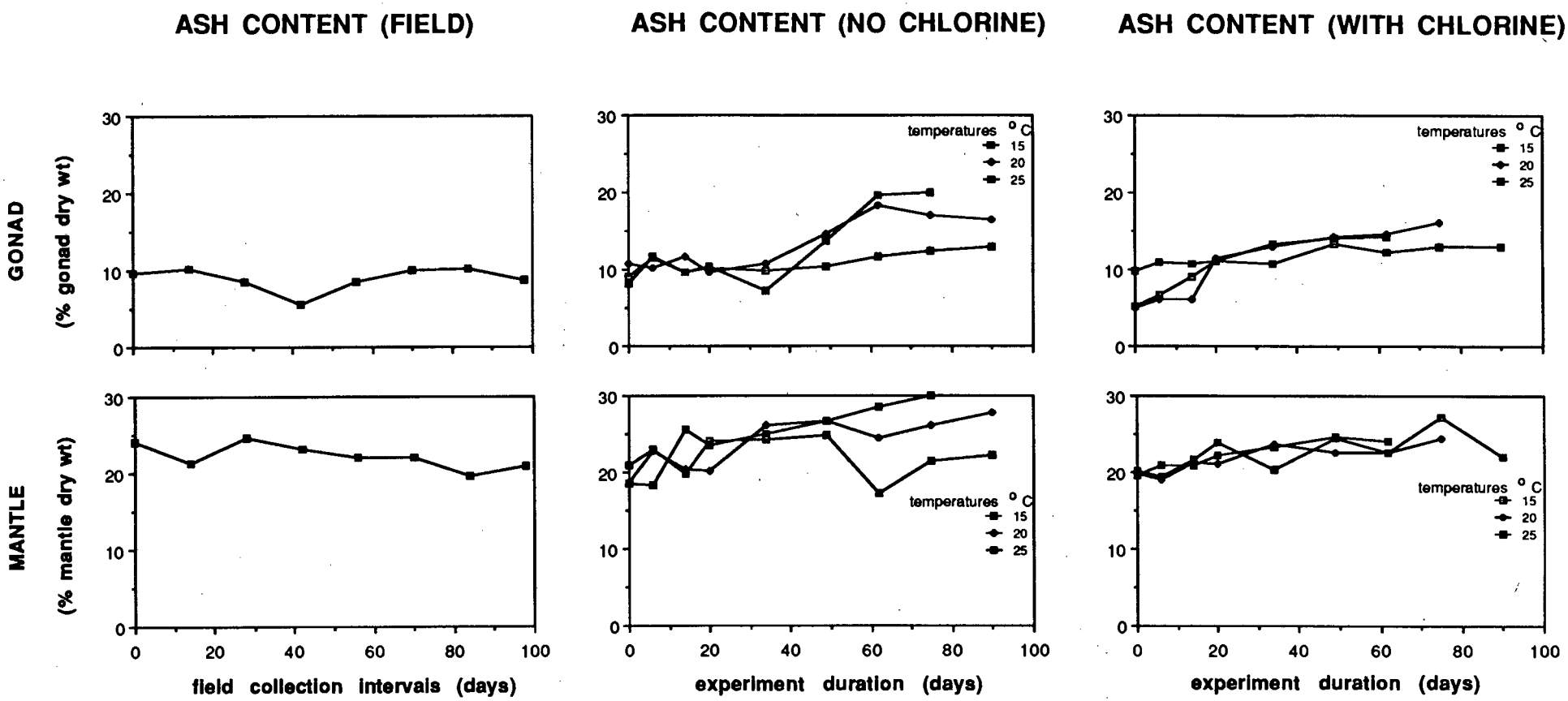

Fig. 3.8. ASH content as percentage of dry weight of gonad and mantle in adult *D. serra* . Animals freshly sampled from the field are compared with those exposed to temperatures of 15, 20 & 25 °C without and with chlorine (0.1 - 0.3 ppm) for a period of 90 days. Each data point is the mean of 2 values.

GAMETOGENIC STAGES

As outlined in the methods, gametogenic stages were arbitrarily denoted by numbers; cytolysed (0), spawned/inactive (1), developing (2) and ripe (3). These stages are displayed in Fig. 3.9 in relation to natural conditions, temperature, chlorine and experiment duration. On each sampling occasion, the gametogenic condition of two separate *D. serra* was established.

Individuals examined immediately after removal from their natural habitat (i.e. day 0) were either in stage 2 or 3 or displayed characteristics intermediate between the two. With prolonged maintenance at 15°C, there appeared to be a shift towards the spawned or inactive (1) and developing (2) stages. Sustained exposure to 20°C evidently increased the occurrence of individuals displaying stage 1 and at 25°C, stages 0 and 1 were the dominant gametogenic conditions. With extended exposure to 25°C, the cytolysed stage became even more common.

The only indication that chlorine had any effect on gametogenic condition at 15°C was the appearance of stages 0 and 1 in some individuals exposed to chlorine for more than 50 days. A combination of chlorine and high temperatures resulted in a marginal shift to stages 1 - 2 from 2 - 3 in the first 20 - 40 days. Thereafter stages 0 - 1 became more prevalent just prior to death.

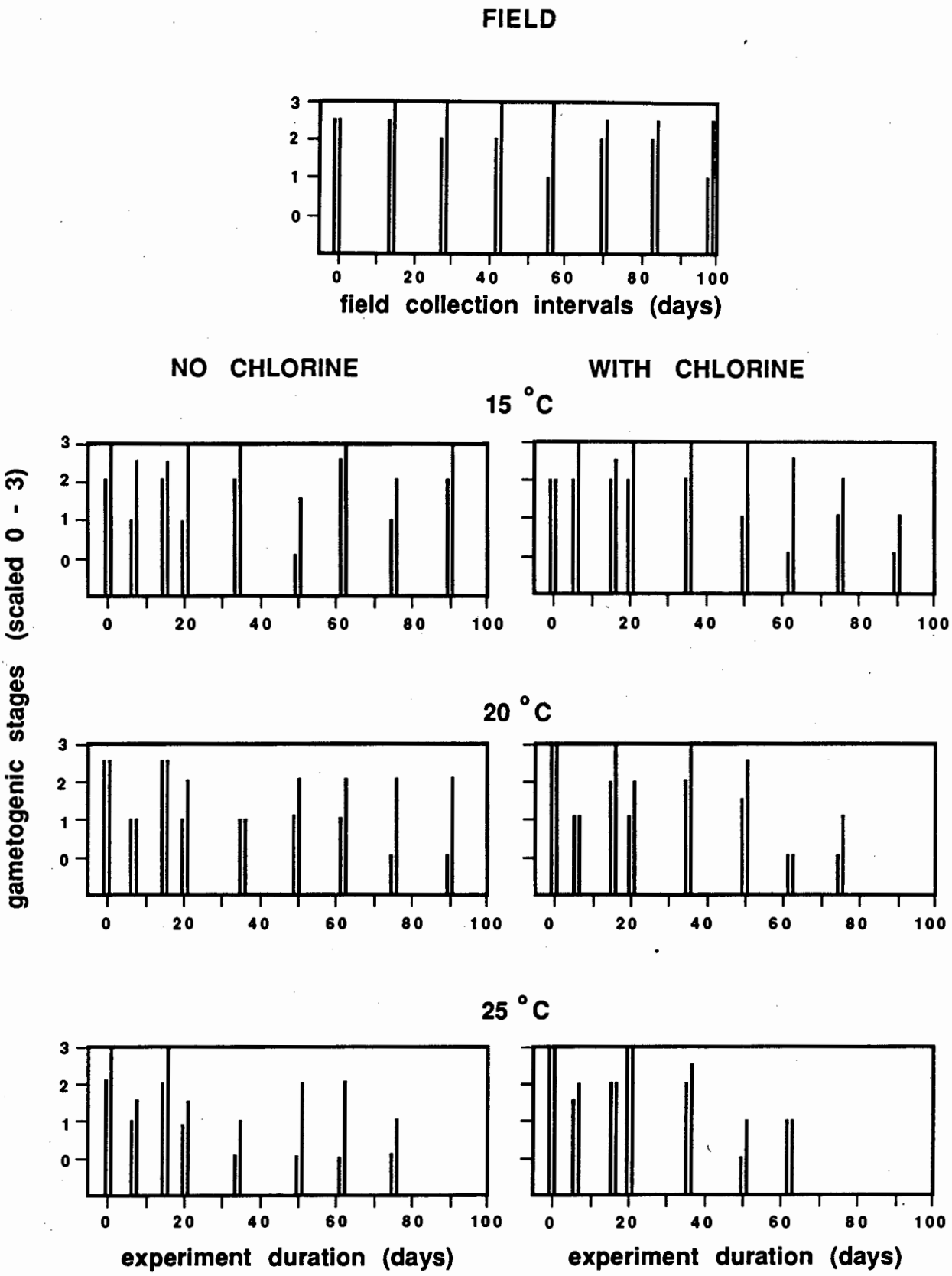

Fig. 3.9. Effect of temperature without and with chlorine (0.1 - 0.3 ppm) on gametogenic stages in laboratory *D. serra* compared with those freshly collected from the field; each sample comprised two individuals (adjacent vertical bars). Cytolysed (0), spawned/inactive (1), developing (2) and ripe (3). Fractions refer to intermediate stages.

CONDITION INDICES

Representation of the overall condition of experimental animals is given in Figs. 3.10 and 3.11 as ratios of tissue dry weight to shell dry weight and volume respectively. Larger animals had proportionately more shell mass than smaller ones. This indicates that mass increments of shell growth exceed that of body tissue during the lifespan of *D. serra*. Much of the weight gain in the shell can be attributed to marked thickening around the umbo and the mantle ridge. By comparison the valves of small *Donax* are paper-thin.

Since shell weight and volume were assumed to remain near-constant, a diminishing index value signifies a loss of body weight. All sizes suffered a decline in body condition with an increase in temperature to 20°C and even more so with a further increase to 25°C. Weight loss at these temperatures was attributable to the loss of protein and carbohydrate reserves from both whole body tissue (Figs. 3.1- 3.3) and from the gonads and mantle in adults (Figs. 3.5- 3.7).

Dosing with chlorine at 15°C resulted in a further small decline in condition with time. At 20 and 25°C on the other hand, chlorine had no additional negative effect on body condition.

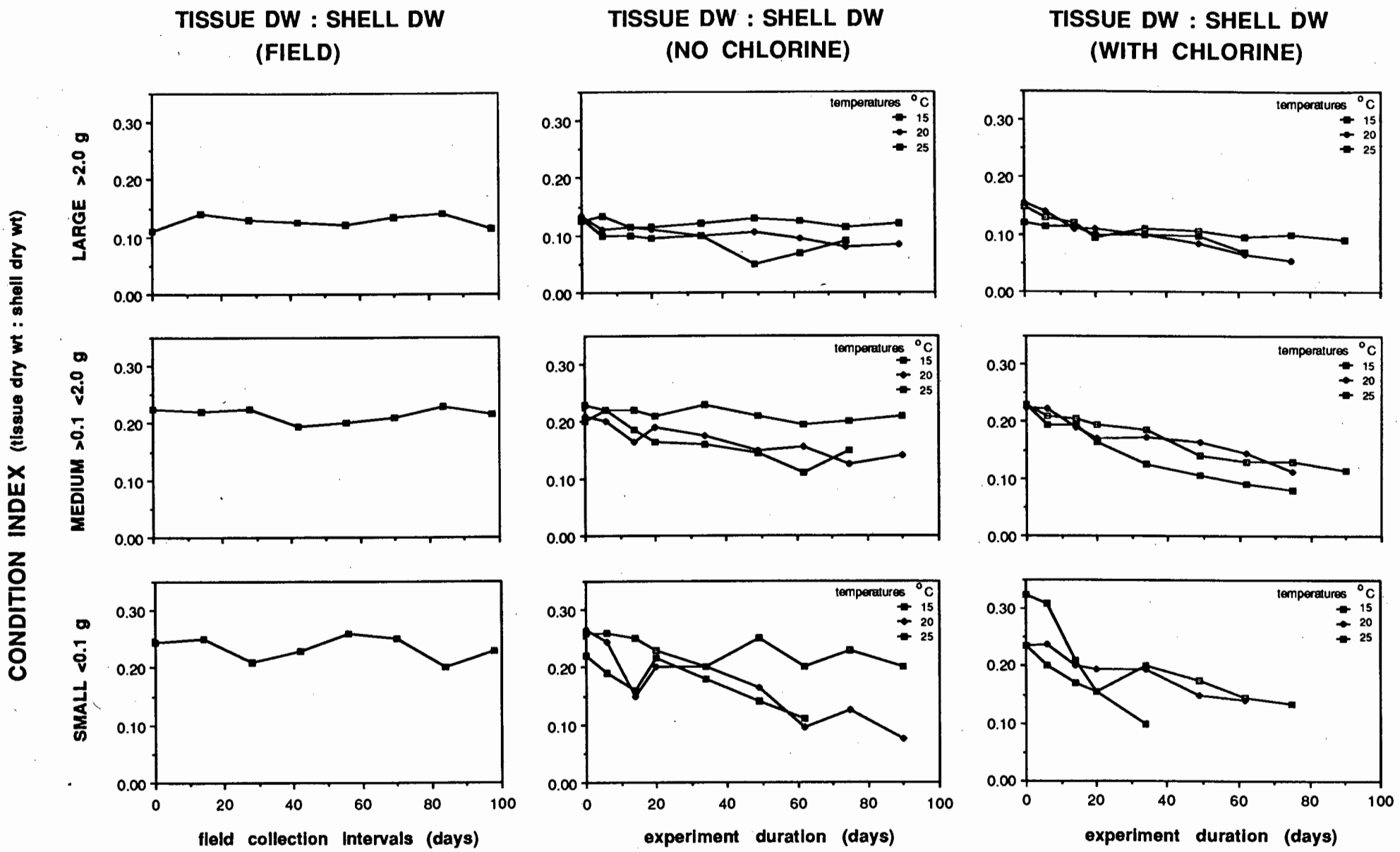

Fig. 3.10. CONDITION INDEX as a ratio of dry tissue weight (g) to dry shell weight (g) in three size groups of *D. serra*. Animals freshly sampled from the field are compared with those exposed to temperatures of 15, 20 & 25 °C without and with chlorine (0.1 - 0.3 ppm) for a period of 90 days. Each data point is the mean of 2 values.

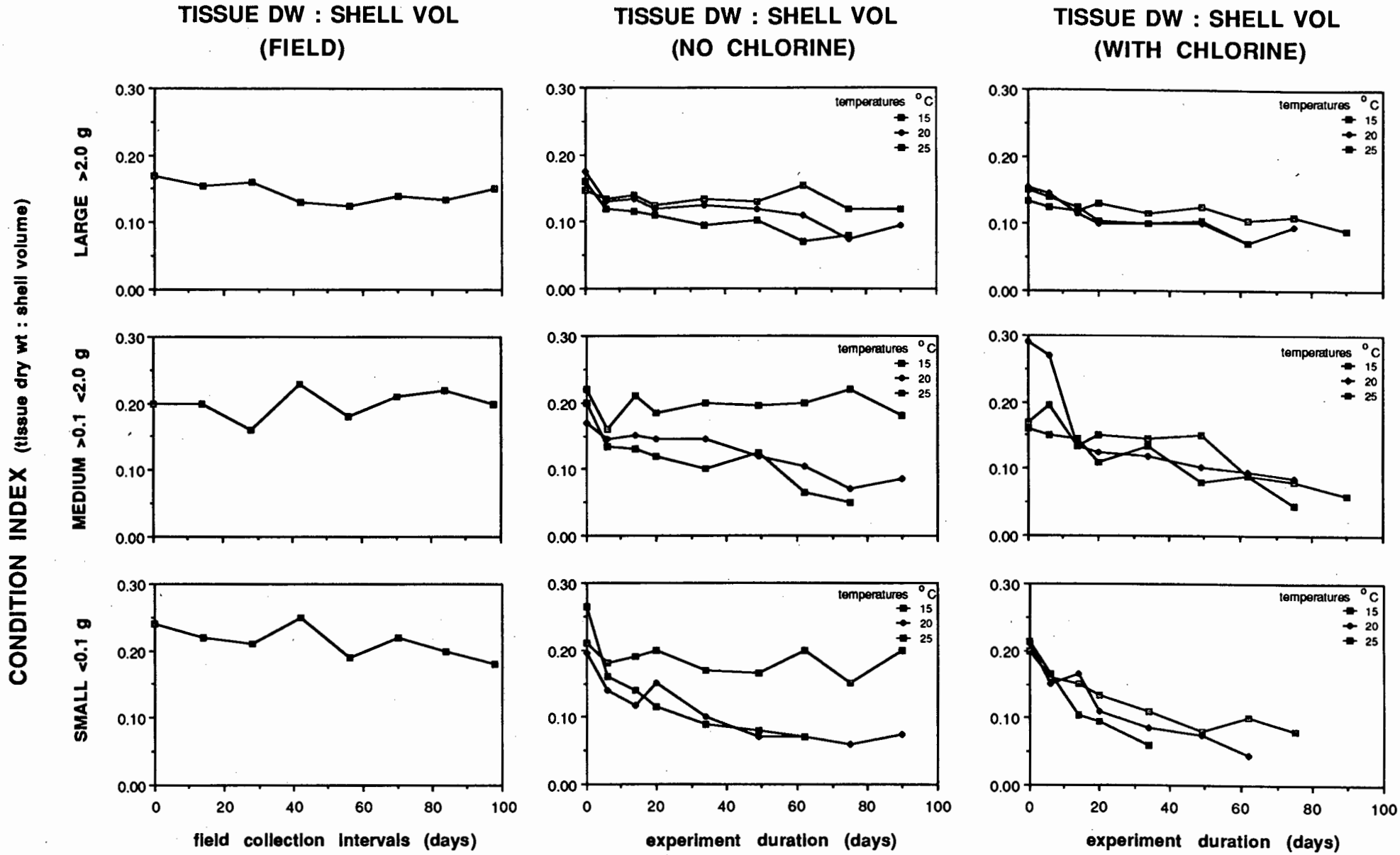

Fig. 3.11. CONDITION INDEX as a ratio of dry tissue weight (g) to shell volume (ml) in three size groups of *D. serra*. Animals freshly sampled from the field are compared with those exposed to temperatures of 15, 20 & 25$_o$ C without and with chlorine (0.1 - 0.3 ppm) for a period of 90 days. Each data point is the mean of 2 values.

DISCUSSION

NATURAL BIOCHEMICAL COMPOSITION AND LABORATORY CONFINEMENT

Similarity between the biochemical composition of freshly-collected animals and those kept under simulated ambient conditions over a period of 3 months demonstrated that prolonged laboratory confinement did not bring about any significant compensatory utilisation of nutrient reserves. There was a small fall in protein content after 20 - 30 days and generally a greater variability in laboratory data, but other than these differences, it was clear that reserves were maintained at natural levels by direct assimilation of the mixed algae-detrital diet. Laboratory controls at 15°C were therefore effective yardsticks against which the effects of temperature and chlorine could be assessed.

In all sizes of *D. serra*, protein was the main constituent (60 - 80%) of whole body tissue, the gonad and mantle. By comparison, total carbohydrate comprised less than 10% of dry weight, mostly in the form of FRS.

Protein content of whole body tissue was similar to that found in *D. serra* on the south coast of South Africa (McLachlan & Hanekom, 1979), but somewhat higher than in northern-temperate *Donax* species (Ansell 1972; Ansell *et al.*, 1980c). On the other hand, the range in carbohydrates (5 - 9%) was below that recorded in these other studies. Where high carbohydrate content (20%) has been recorded, it is associated with vitellogenesis (pre-spawning) whilst

minima (6%) occur after spawning. Such a wide range represents the extremes of a regular pattern associated with seasonal reproductive cycles.

Unlike protein and carbohydrates, lipid content in south-coast *D. serra* did not follow an annual cycle, remaining between 3 and 5% of dry weight. As 85 - 95% of whole body reserves in west-coast *D. serra* were comprised of protein and carbohydrate, it is reasonable to suggest that lipid levels (% of dry weight) in these animals are similar to those of south-coast individuals.

The very high organic content of gonads in this study (90 - 95%), to which protein and carbohydrate contributed no more than 70%, may signify that this tissue is an important site for lipid storage, a reserve noted for its high calorific value (Ansell, 1974a; Gabbott, 1975; Castro & Mattio, 1987). Among marine bivalves in general, gonads are rich in lipids, especially when the eggs are mature and ready for release for fertilization and planktonic development (Ansell, 1974b; Bayne *et al.*, 1975; Pieters *et al.*, 1980, Epp *et al.*, 1988).

Unlike south-coast *D. serra* and other *Donax* species, west-coast individuals have an ill-defined reproductive cycle. Although there are weak indications of prolonged spawning periods from May to November and again from February till March (de Villiers, 1975a; Birkett & Cook, 1987), at any specific time there can be considerable individual variation in gametogenic state. Variation

observed in gonad reserves over 3 months in this study is probably a reflection of this individuality. Indeed, fresh gonad smears indicated that all major gametogenic states (cytolysed, inactive/spawned, developing and ripe) could be identified among field and laboratory-control individuals. With an unpredictable reproductive pattern, which may be linked to the lack of strong seasonal changes in temperature and food availability (see Chapter 4), biochemical content of adults estimated from April to June is likely to be fairly representative of other times of the year.

Carbohydrate composition in *D. serra* contrasted even more sharply with that of mytilid species. In these bivalves, carbohydrate has been found to reach 35% of dry weight prior to spawning, falling to 4 - 10% post-spawned (De Zwaan & Zandee, 1972; Dare & Edwards, 1975; Zandee *et al.*, 1980). Even in *D. serra* identified as being in the pre-spawned condition, carbohydrate content did not exceed 10%. Glycogen comprised 90% of total carbohydrate in *M. edulis;* in *D. serra* the largest carbohydrate subfraction was free reducing sugars in both whole and fractions of tissue. By difference, this means that only a small proportion of carbohydrate was stored as a long-term energy reserve in the form of glycogen. FRS would only provide energy for immediate needs such as burrowing, surfing and maintenance of position in the intertidal zone.

Under stress it was evident that the demands of increased metabolic rates (see Chapter 7) were mostly met by

catabolising protein, the major source of stored energy in *D. serra*. The mantle and to a lesser extent, gonads were clearly important protein storage sites. The foot and adductor muscles are also major potential storage sites. This assumes that the 60 - 80% protein content of total dry weight originates primarily from the foot and adductors which together make up 70 to 80% of overall weight.

In support of this, studies on pectinids (e.g. *Chlamys septemradiata, Argopecten irradians*) have shown that protein stored in the large adductor muscles is the main energy substrate for locomotion, gametogenesis and growth (Ansell, 1974b; Taylor & Venn, 1979; Barber & Blake, 1983; Epp *et al.*, 1988). From these findings it is tempting to postulate that only in bivalves possessing extensive locomotor muscle tissue is protein content sufficient to constitute the main metabolic energy source. The importance of protein as an energy source for muscular activity in the foot of *D. serra* has been indicated by the work of Blackstock & Ansell (1981). It was found that *D. serra* had exceptionally elevated octopine dehydrogenase activity in the foot. These authors implied that ATP for muscular contraction is obtained largely by the breakdown of a protein-based phosphagen, arginine phosphate.

Conserving protein as the main energy source may hold metabolic disadvantages as well as advantages for *D. serra*. Protein is energetically more expensive to catabolise than either carbohydrate or lipid (Gabbott, 1983). Furthermore,

the ammonia released from protein breakdown is toxic when accumulated in muscle tissue. However, the one major advantage is that protein, unlike lipid, can be catabolised aerobically and anaerobically (carbohydrate holds the same advantage while lipid can only be utilised aerobically) (Holland, 1978). Substantial anaerobic respiration has been indicated in *D. serra*, especially during heightened burrowing activity (Trueman & Brown, 1987; van Wijk *et al.*, 1989) and when stressed (Chapter 5).

EFFECTS OF TEMPERATURE AND CHLORINE

Conservation of protein and carbohydrate under normal conditions indicated that *D. serra* was able to obtain sufficient energy from external food sources rather than from reserves to sustain metabolism. Such conservation depends on the rates of catabolism being less than anabolism.

In this study exposure to high temperature and chlorine resulted in catabolism markedly exceeding anabolism. Reserve depletion indicated that energy intake could not offset the considerable metabolic stress in *D. serra*. Furthermore, since the quantity of biochemical constituents declined continually throughout the 90-day experiment, it appears that at no time did *D. serra* acclimate to the imposed conditions. Rates of oxygen consumption and ammonia excretion confirm this (Chapter 7). These rates of

metabolic expenditure remained raised at 20 and 25°C, even after 14 days.

The most striking indication of the vulnerability of *D. serra* to chlorine was demonstrated by the marked depletion of reserves, especially protein, at normal temperatures (15°C). At 20 and 25°C, the presence of chlorine did not generally result in further use of carbohydrates, but there was extra loss of protein in whole body tissue and the gonads. Overall, protein was utilised more extensively and more readily than carbohydrate as an energy source during stress. Enhanced protein catabolism was also indicated by increased rates of ammonia excretion and a decrease in O:N ratios on exposure to raised temperature and chlorine (see Chapter 7).

Other studies concerned with changes in biochemical composition of marine bivalves have concentrated on the effects of natural stress factors such as sharp seasonal changes in temperature and food availability (review Gabbott, 1983). In many cases naturally unfavourable conditions induced similar depletion of reserves as observed in *D. serra* exposed to atypical stresses. In *D. vittatus* for example, prolonged starvation led to a rapid increase in protein catabolism accompanied by increased ammonia excretion and a fall in O:N ratios (Ansell & Sivadas, 1973). In *M. edulis*, overwintering stress resulted in rapid utilisation of stored reserves, especially carbohydrate and protein (Gabbott & Bayne, 1973; Dare & Edwards, 1975; Zandee

et al., 1980). As observed in *D. serra*, this species displayed distinct gonad recession under prolonged stress and this, together with reserve depletion also led to a loss of body weight (Bayne & Thompson, 1970; Bayne, 1975; Pieters *et al.*, 1980).

In *D. vittatus* (Ansell & Sivadas, 1973) and *Tapes japonica* (Mann & Glomb, 1978), exposure to high temperatures led to a similar weight loss, gonad recession and reserve depletion as observed in *D. serra*. As far as is known, there has only been one previous investigation into the effects of chlorine and this only considered changes in the gonad and condition indices at ambient temperature. In that study the oyster, *Crassostrea virginica*, on exposure to between 0.1 and 0.3 ppm, displayed the same decline in body condition but an even more advanced degradation of the gonads than observed in *D. serra* (Scott & Middaugh, 1978).

Regardless of the origins of stress, it is clear that marine bivalves generally resort to stored reserves to meet the cost of compensatory increases in energy expenditure. Although the nature of the reserve most utilised under stress may differ (e.g. glycogen in *M. edulis*, protein in *D. serra*), the end result is the same; weight loss and after prolonged stress, gonad recession followed by death.

It is important to view the results in this study in the context of conditions at the outfall of the power station. Obviously, in this environment it is unlikely that *D. serra* would be continuously exposed to high temperatures

and chlorinated water for 90 days. At most, conditions imposed in the present experiments could only persist for 4 to 5 days during strong on-shore north-westerly winds (see Chapter 1, Fig. 1.2). For instance, over this time period at a temperature of 20°C, gross protein composition of adults could be expected to fall by about 20% and that of carbohydrates by 1 to 2%. Juveniles, although more susceptible to sustained elevated temperature and chlorine (see also Chapter 2), have added protection by residing in the intertidal zone and only being exposed to contaminated sea water tidally. Individuals likely to be most affected would be those directly in the path of the effluent, that is within 20 to 40 m of the outlet. However, even in this area, dislodgement from the sand by the strong outfall current is a greater danger than the progressive depletion of reserves as a result of effluent temperature and chlorination.

CONCLUSIONS

1.) Prolonged laboratory confinement in which fresh sea water at ambient temperature and a mixed diet of algae and natural detritus were frequently provided, had no significant detrimental effect on the biochemical composition of all sized *D. serra*.

2.) Since *D. serra* at Ouskip has an ill-defined reproductive cycle, it is supposed that a seasonal pattern

in body reserves, so distinct in northern-temperate bivalve species, would be unlikely.

3.) Protein, rather than carbohydrate appeared to be the major energy reserve. The most likely sites for storage of this reserve are the mantle, foot and adductor muscles. Since *D. serra* often functions anaerobically, it is a metabolic advantage to have protein as the major reserve as it can be used both aerobically and anaerobically.

4.) The continual loss of reserves throughout 90 days of exposure to high temperatures and chlorine, even with food provided, was believed to indicate non-acclimation to these atypical conditions. Progressive degeneration of the gonads was closely coupled with the depletion of body and reproductive reserves of protein and carbohydrate.

5.) The effect of chlorine on biochemical composition was most evident at ambient temperature. In combination with high temperatures, the effect of chlorine was less marked but still apparent in the further loss of protein.

6.) Conditions at the outfall of Koeberg Nuclear Power Station are unlikely to cause long-term depletion of biochemical reserves in any size of *D. serra* since exposure to temperature and chlorine levels as used in the present study would only be intermittent and of short duration.

S E C T I O N II

PHYSIOLOGICAL ENERGETICS

CHAPTER FOUR

INTERACTIVE EFFECT OF BODY SIZE AND DIET ON CLEARANCE, INGESTION AND ABSORPTION RATES

INTRODUCTION

The nutritional biology and energetics of marine filter-feeding bivalves have received much attention as reviewed by Bayne (1976), Bayne & Newell (1983) and Griffiths & Griffiths (1987). Most of this research has focused on computing energy budgets using varied concentrations of cultured unicellular algae. These studies contributed significantly towards understanding the fundamental principles of feeding energetics and their relationship with food quantity. It has since been recognised however, that food quality, especially as it relates to the characteristics of natural particulates, must also be considered.

Attempts have been made to synthesise laboratory diets resembling ambient particle size spectra, organic:inorganic ratios, calorific values and biochemical composition. Mixed-algae suspensions, sometimes in combination with bacteria (Stuart & Klumpp, 1984; Bayne *et al.*, 1984; Cucci *et al.*, 1985; Shumway *et al.*, 1985; Amouroux, 1986), or mixtures of natural silt and cultured algae have been used for this purpose (Kiorboe & Mohlenberg, 1981; Kiorboe *et al.*, 1981; Mohlenberg & Kiorboe, 1981; Newell & Jordan, 1983; Bricelj & Malouf, 1984; Robinson *et al.*, 1984; Bayne *et al.*, 1987; de Villiers & Allanson, 1988).

Of greater ecological significance however, are laboratory experiments using natural food materials. Dried and powdered saltmarsh leaves served as food in studies on

digestion and absorption in *Crassostrea virginica* (Lucas & Newell, 1984; Newell & Langdon, 1986), whilst Stuart (1982) fed the kelp-bed mussel *Aulacomya ater* kelp detritus derived from macrophyte fronds which included epiphytic bacteria. Even more meaningful are experiments in which bivalves are maintained in unfiltered sea water collected from the natural environment (Wright *et al.*, 1982; Newell & Jordan, 1983; Lucas *et al.*, 1987; Matthews *et al.*, 1989). This provides a natural assemblage of bacteria, phytoplankton, detritus and resuspended faeces as a food resource.

Most commonly used quality criteria are calories/Joules, mg organics, mg carbon and mg nitrogen per unit dry weight of particulates. Tenore (1981) and Hanson (1982) recommended that the energetic value of food should be further refined by regarding only that fraction which can be hydrolysed by HCl in the stomach. In a study on feeding and digestion by *M. edulis*, Bayne *et al.* (1987) found a better correlation between absorption efficiency and food quality when the latter was expressed as organics per unit volume of particles rather than per unit dry mass so highlighting the volumetric constraints associated with feeding.

In some cases certain quality criteria are chosen to aid the interpretation of specific physiological processes. For instance, the proportionality of carbohydrates, proteins and lipids is more important than organic or energy content with regard to growth in juvenile bivalves (Flaak &

Epifanio, 1978; Wikfors *et al.*, 1984; Mayasich & Smucker, 1986). Calculation of digestive efficiencies relies on knowing the chlorophyll *a* value of food as well as faeces (Robinson *et al.*, 1984; Hawkins *et al.*, 1986) and organic content is essential in the application of the well-known ash-ratio method (Conover, 1966). Trophic relations and resource transformations of bivalves are often expressed in terms of organic carbon and/or nitrogen content (Seiderer *et al.*, 1982; Stuart *et al.* 1982a, b; Hawkins & Bayne, 1985; Seiderer & Newell, 1985; Lucas *et al.*, 1987; Matthews *et al.*, 1989) so that C:N ratios become significant quality criteria. However further expressions of quality may be required, since maintenance requirements computed in elemental terms will change according to the digestibility, biochemical composition and energy content of available food.

Another important aspect often neglected in nutritional studies is an attempt to link laboratory feeding data to measures of the availability of and seasonal variation in natural resources. Most research in this respect has been conducted on *Choromytilus meridionalis* and *A. ater* in the context of the kelp-bed ecosystem off the south-western Cape of South Africa (Griffiths & King, 1979a; Griffiths, 1980b; Stuart & Klumpp, 1984; Bayne *et al*, 1984; Seiderer & Newell, 1985). Besides these studies, Bayne *et al.* (1987) simulated seasonal changes in natural seston concentrations by adding silt to cultured algae in their work on *M. edulis*.

This chapter endeavours to show seasonal changes in the quantity and quality of suspended particulates in the surf water at Ouskip and to highlight differences in feeding on artificial compared to natural foods. Cultured alga, *Tetraselmis suecica* and detrital foam collected after stranding on Ouskip beach were used to provide this contrast. The range of laboratory food concentrations was chosen to match ambient changes in quantity (particle numbers/volume l^{-1} and dry weight l^{-1}). Quality criteria were set at percentage organics, energy content, organic carbon to nitrogen and organic carbon to chlorophyll a ratios and percentage organics per unit particle volume. Effects of dietary quantity and quality on size-related rates of clearance, ingestion and absorption in *D. serra* were investigated.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

D. serra were collected at low water spring tides (LWS) from Ouskip and kept with sand at 15°C in 30-l tanks with flowing, aerated sea water. All animals were acclimated to laboratory conditions for one to two weeks before being used in experiments. During this time they were fed a mixed diet of *Tetraselmis suecica, Tetraselmis chuii, Dunaliella primolecta* and detrital foam.

All of the three major growth cohorts in the population, namely <7 mm, >7 <35 mm and >35 mm shell width, were represented (see Chapter 2). At the end of an experiment, individuals were dried at 60°C for 2 days to determine the weight of soft tissue. A portion of dried tissue was combusted in a bomb calorimeter for energy content which meaned at 17.336 J·mg dry weight^{-1} ($\pm$1.032, n=10). In some instances, the relationship between shell width and dry tissue weight (DW) was obtained from regressed data (see Chapter 6).

SEA WATER ANALYSIS

Estimates of the quantity and quality of particulate matter in sea water at Ouskip were obtained by sampling in the surf zone at monthly intervals from March, 1985 to May, 1986. At the time of LWS, 25 litres of sea water were collected and the suspended particulate material analysed immediately on return to the laboratory. Particle concentration, dry mass, organic, energy and carbon content, as well as chlorophyll *a* were determined.

Particle concentration, dry mass, organic and energy content

Total volume and counts of particles, plus the size distribution of these particles were determined on a Model TA II Coulter counter using both the 70 um and 280 um aperture orifice tubes. This allowed the counting of particles from 1.26 um to 50.8 um in diameter. However, since over 90% of surf particles were <20 um, counts were

restricted to below this particle diameter. Those <3 um were generally excluded since electronic interpretation of numbers in this size fraction by the Coulter counter was unreliable. In most samples 9 size categories based on particle diameter were distinguished and the geometric mean volume and number in each category were used to calculate particle volume (Strickland & Parson, 1972). Data were finally expressed per litre of sea water following extrapolation from the mean count of 3 X 2-ml samples analysed on the Coulter counter.

Six 300-ml samples of surf water were filtered onto ashed pre-weighed 25-mm Whatman GF/C filters which have a retention efficiency greater than 80% for particles >2 um diameter (Sheldon, 1972). The filters were rinsed with isotonic ammonium formate to remove salt, dried at 60°C for 2 days and then weighed to estimate the dry mass of the particulate material (PM) l^{-1}. The filters were then combusted at 480°C for 5 hrs and weighed again to obtain, by difference, the mass of particulate organic matter (POM).

The energy value of PM and POM was determined by filtering 15 l of sea water through a 1-um Nuclepore membrane filter, rinsing with distilled water and then gently scraping the residue onto a glass slide. Once freeze-dried for 3 days, the residue was analysed in triplicate using a microbomb calorimeter.

C:N ratios

A further six 300-ml samples were screened through a 100-um mesh and filtered onto ashed Whatman GF/C filters which were then fumed over concentrated HCl to drive off inorganic carbon. Following freeze-drying, the organic carbon and nitrogen content of the samples were measured by combustion in a Heraeus CHN Analyser. Results were expressed as ratios of carbon to nitrogen as well as mg dry weight per litre of surf water.

Chlorophyll a

Three 1-l samples were filtered onto 45-mm Whatman GF/C filters and then frozen for up to one week prior to analysis. Pigment was extracted in 10 ml of 90% acetone and the optical density determined on a spectrophotometer at 630, 645, 665 and 750 nm. Chlorophyll a concentrations were calculated according to the trichromatic equations given by SCOR/UNESCO (1966).

FOOD PREPARATION

The dinoflagellate *Tetraselmis suecica* (Kylin) [5 to 8 um in diameter] was cultured in autoclaved conical flasks containing 2-l filtered (0.45 um), sterilised sea water and 2 ml of Walne's growth medium. The cultures were continuously aerated and maintained at 12°C below a bank of 3 double 20 W fluorescent lights (Agro-lite) with a 14:10 photoperiod until in their logarithmic phase of growth when they were used in experiments. This procedure was followed

CLEARANCE AND INGESTION RATES

For each experiment, which lasted 8 - 10 hrs, animals representing a wide size range were placed in 8 separate 2-l glass beakers filled with 600-ml sand and 1-l 0.45 um filtered sea water at 15°C. Each beaker contained either 1 large mussel (>35 mm), 2 medium sized individuals (>7 <35 mm) or 4 small ones (<7 mm). Prior to use, the sand was thoroughly rinsed in distilled water followed by 0.45 um filtered sea water to remove extraneous particles which could add to the background particle count in the beakers. The sea water in the beakers was gently aerated and circulated by means of air-lift pumps fitted just above the sand. Before experiments commenced, animals were maintained for 24 hrs in 0.45-um filtered sea water to purge their digestive tracts of faecal material. Animals were then transferred to the beakers and allowed to equilibrate for 2 hrs before adding algae or detrital foam.

There has been some confusion in recent literature on the definition of clearance rate. The rate is sometimes defined as the number of food particles cleared from a litre of sea water hr^{-1} (Shumway *et al.*, 1985), but is more commonly used synonymously with filtration or irrigation rate to describe the volume of water cleared of particles hr^{-1} (Bricelj & Malouf, 1984; Hawkins *et al.*, 1985; Bayne *et al.*, 1987; Lucas *et al.*, 1987; Matthews *et al.*, 1989). The latter definition is adopted here.

Clearance rates were determined indirectly by measuring the decline in a known concentration of algal cells or detrital particles per unit time. Experiments were conducted at the following ration levels: *T. suecica* - 5, 10, 25, 30, 35, 40 and 50 X 10^6 cells l^{-1}, or 1.25 to 12.45 mg DW l^{-1}; detrital foam - 5, 10, 20, 30 and 40 X 10^6 particles l^{-1}, or 1.37 mg DW to 10.92 mg DW l^{-1}. After adding appropriate volumes of food, 5-ml samples were removed every 20 mins to follow the decline in particle numbers. Additional food was added at these intervals to maintain levels within 20% of the desired concentration over a period of 8 hrs. Results were corrected for particle settlement and algal growth using control beakers containing food and sand only, while beakers without food but containing animals and sand in filtered sea water were used to correct for any production of particles by the animals.

The rate of change in particle concentrations in controls was obtained from the following equation:

$$a = \frac{\log_e \text{conc}_o - \log_e \text{conc}_t}{t}$$

where a = rate of change in particle concentration; conc_o = initial concentration at time 0; conc_t = concentration after time t in hours. Clearance rates were calculated for each

20 min period according to the standard formula (Coughlan, 1969):

$$\text{Clearance rate } (1\ h^{-1}) = \frac{V}{N} \times \left(\frac{\log_e \text{conc}_1 - \log_e \text{conc}_2}{t} - a \right)$$

where conc_1 = particle concentration at time t_1; conc_2 = particle concentration at time t_2; t = elapsed time in hours; V = volume of sea water in liters; N = number of animals per beaker; a = control. An entire experiment provided 10 to 14 estimates of clearance rates which were averaged for each individual or group of animals. Estimates from the first hour were ignored as this was regarded as a period of adjustment to the presence of food following a 24-hr period in particle-free water.

An indirect measure of clearance rate as described above has received much criticism, mainly because it employs a static system in which the ration level is continually changing and excretory products accumulate, thereby introducing uncontrolled variables in rate measurements (see review by Winter, 1978). Flow-through systems in which the above variables are eliminated are therefore favoured. However, preliminary experiments using such a system with a capacity of 30 l provided similar results. As a precautionary measure in the static set-up, three quarters of the sea water in a beaker was siphoned off and replaced very slowly every 2 hrs with sea fresh water containing a particle density at the desired concentration. This did not

disturb filter feeding activity as long as the animal's siphons were not touched or exposed.

Ingestion rate (IR), as particle numbers ingested h^{-1} $individual^{-1}$, was calculated from clearance rate (l h^{-1}) X <C>, the mean particle density (i.e. $\{conc_1 + conc_2\}/2$). Frost (1972) provides a far more complicated formula to calculate mean concentrations, but the simple computation used here always provided an answer within 1% of his calculation. Measures of the dry weight of a known concentration of algae and detritus and of the experimental animal were used to convert IR to mg DW ingested g dry tissue $weight^{-1}$ h^{-1}.

ABSORPTION EFFICIENCY AND RATES

The efficiency with which *T. suecica* and detrital foam were absorbed was measured simultaneously with clearance rates using the ash-ratio method of Conover (1966). Faeces produced within the first 2 hrs were discarded as a precaution against including excretory products from a previous meal. As faecal strings emerged from the exhalent siphon, they were immediately collected and accumulated for each individual in a small volume of sea water. This procedure eliminated the possibility of underestimating clearance rates by inadvertently increasing the number of particles by fragmentation and resuspension of faeces (see Hildreth, 1980). At the end of a run, faeces were gently rinsed in isotonic ammonium formate to remove salts and

adhering sand grains and then filtered onto pre-ashed, weighed, glass fibre filters under low vacuum. In addition, triplicate samples of known volumes of algal and detrital suspension from current stocks were filtered.

All filters were dried at 60°C for 2 days, weighed on a Mettler ME30 microbalance, ashed at 450°C for 5 hrs and then re-weighed. The organic fraction of faeces was compared with that of food by means of the Conover ratio:

$$\text{Absorption efficiency (AE) \%} = \frac{f - e}{(1 - e)f} \times 100$$

where f is the organic fraction of food and e is the same for faeces. AE measured by this method refers to net absorption as it only takes into account the efficiency with which ingested food is absorbed across the gut wall and ignores excretory products such as mucus (Bayne *et. al.*, 1984; Hawkins & Bayne, 1984). The net absorption rate (AR) is thus derived from IR X AE.

RESULTS

SEA WATER ANALYSIS

Monthly estimates, for a period of 15 months, of the quantity and quality of particulate suspended material in surf water at Ouskip are summarised in Table 4.1 for a period of 15 months. Quantity was expressed as concentration of particles (total counts and volume), dry weight (DW), energy content, organic carbon and chlorophyll *a* concentration. Quality was expressed as % organics, C:N and organic C:Chlorophyll *a* ratios.

Although data were collected only once each month, some trend can be inferred. Data means show that particulate numbers, volume, dry mass, energy and organic carbon were greatest during conditions more typical of winter (onshore NW winds). This was especially so when accompanied by 2 to 3 metre swells as prevailed in April 1985 when the highest suspension load was measured (25.28 mg DW l^{-1}). Large quantities of seafoam were present on this occasion. Chlorophyll *a* levels in this foam concentrate reached 508.60 *ug* l^{-1} compared to only 10.75 *ug* l^{-1} in surf water below the foam. Although concentrations of seafoam were not evident during offshore SE winds, which are more common in summer, mean chlorophyll *a* in surf water was slightly higher than that calculated at the time of NW winds. Particle concentration and quality can also be related to episodic upwelling (see discussion).

Table 4.1 Quantity and quality of suspended particulates in the surf at Ouskip based on samples collected monthly at LWS. Wind directions are abbreviated to NW = north west, SE = south east and W = westerly and Chl = Chlorophyll. ± SD = one standard deviation from mean. Particle counts and volume include >3 <20 um diameters.

YR/MTH & WIND	QUANTITY						QUALITY		
1985	Particles $10^6 l^{-1}$	mm^3	Dry wt. $mg\ l^{-1}$	Energy content $J\ l^{-1}$	Org. Carbon $mg\ l^{-1}$	Chl a $ug\ l^{-1}$	% Organics	C:N	Organic C:Chl a
3 SE	11.32	1.07	2.22 ±1.20	6.70	0.17	1.01	48.21 ±11.38	8.75	168
4 NW	82.45	23.62	25.28 ±5.90	106.68	2.29	10.75	56.69 ±11.96	8.27	211
5 W	31.57	9.07	9.23 ±1.40	46.70	0.61	3.15	43.14 ±4.37	7.10	193
6 NW	20.13	5.76	5.27 ±1.25	18.60	0.47	2.75	49.95 ±3.78	7.23	167
7 NE	21.03	5.99	6.15 ±0.79	27.37	0.35	1.62	45.73 ±3.12	8.56	216
8 NW	31.04	8.91	9.37 ±0.44	46.94	0.56	2.15	43.21 ±2.30	7.65	260
9 NW	29.25	5.01	8.62 ±0.32	35.77	0.59	2.16	42.11 ±1.02	8.00	223
10 SE	39.36	5.00	11.20 ±2.76	51.18	0.36	7.88	22.12 ±2.21	8.20	46
11 SE	21.00	5.07	6.20 ±0.23	24.92	0.27	7.68	36.91 ±7.80	5.82	30
12 W	62.00	12.59	14.38 ±3.60	63.13	1.01	5.72	37.82 ±16.94	5.93	173
1986									
1 NW	14.69	3.62	4.21 ±1.04	15.79	0.49	1.05	64.00 ±11.09	5.85	390
2 SE	19.34	6.04	6.07 ±1.91	25.80	0.20	5.43	32.01 ±6.52	6.40	44
3 SE	34.56	5.34	8.23 ±0.87	49.46	0.34	4.57	30.91 ±4.09	7.40	72
4 NW	19.50	5.92	6.02 ±0.64	19.56	0.51	3.59	47.14 ±4.50	6.00	142
5 NW	23.88	9.50	7.87 ±1.38	32.50	0.58	7.89	40.00 ±3.13	6.35	74
Mean	30.74	7.50	8.69	38.07	0.59	4.49	42.66	7.17	160
±SD	19.57	5.23	5.65	24.59	0.51	2.97	10.36	1.05	97
NW-									
Mean	31.56	8.91	9.52	39.41	0.78	4.33	49.01	7.05	210
±SD	23.15	6.82	7.19	31.70	0.67	3.59	8.66	0.98	100
SE-									
Mean	25.12	4.50	6.78	31.61	0.27	5.31	34.03	7.31	72
±SD	11.54	1.96	3.29	18.71	0.08	2.80	9.55	1.22	56

In all samples 96 - 99% of particles counted were <20 um. Densities of particles of 3 to 20-um diameter fluctuated from 11.32 X 10^6 l^{-1} to 82.45 X 10^6 l^{-1}, corresponding to particle volumes of 2.22 to 23.62 mm^3 l^{-1}. Examples of particle-size distribution under different wind conditions are shown in Fig. 4.1, where particle volume is plotted against $\log_{10}$ of the mean particle diameter over the range 1.26 to 50.8 um. During NW winds, particulate densities were 3 to 4 times higher than during SE winds, and incorporated a greater proportion of large (>20 um) particles.

Microscopic inspection of surf water revealed particulate aggregations made up of tiny sand granules, intact and fragmented phytoplankton cells, zooplankton exuviae, larvae, faecel pellets and sandy-beach nematodes. The dominant phytoplankton genera were *Chaetoceros, Skeletonema, Rhizosolenia, Ceratium* and *Thalassosira.* Most of the aggregations however, consisted of amorphous, yellow-brown, irregular-shaped particles, as well as spherical droplets with an "oily" appearance which suggests a possible origin from films of dissolved organic matter. Such aggregations were more prevalent during NW winds.

Quality of particulates (% organics, and ratios of organic C:N and C:Chlorophyll *a*, Table 4.1), indicates the organic portion of the food resource which is of most nutritional value to *D. serra.* Organic content was low (43% on average), but this is to be expected in a wave-exposed

environment with a naturally high silt load. Energy content of particulates showed little variation over 15 months (4.25 $\pm$ 0.75 J mg^{-1}). There was a significant negative correlation between organic and energy content of particulates expressed by the equation: J mg^{-1} = 5.75 - 0.04 X %organics [r = -0.49; n = 15, P > 0.05]. C:N ratios were consistent within the range 5.8 to 8.8. This is indicative of phytoplankton-derived detritus conforming to the Redfield ratio of 6.5 (Redfield *et al.*, 1963). The most striking contrast in particulate quality is revealed by C:Chlorophyll *a* ratios under different wind conditions. The high value of 210 is indicative of a greater concentration of detritus in surf when NW winds prevailed compared to a ratio of 72 during SE winds reflecting dominance of live and decaying phytoplankton cells (Banse, 1977; Andrews & Hutchings, 1980).

COMPARISON BETWEEN LABORATORY AND NATURAL FOOD

Table 4.2 summarises conversions between particle counts and volume, dry mass, % organics, energy values and carbon and nitrogen content of the two laboratory foods. There was little difference between the dry mass of 10^6 algal cells and 10^6 detrital particles. In terms of quality, carbon content and C:N ratios were also similar, but percentage organics and energy content per mg DW was higher in algae. As a unit volume of material however, seafoam detritus was the greater in organic and energy content. This has

Table 4.2. Conversions between particle counts and volume (mm^3), dry mass, percentage organics, energy values and carbon content of the two laboratory foods *T. suecica* and detrital foam. DW = dry weight, POM = particulate organic matter, C = carbon. Values in brackets represent number of samples, n; standard deviation, ±SD).

	T. suecica	detrital foam
mg DW·10^6 particles^{-1} >3 <20 um	0.249 (14; 0.018)	0.273 (14; 0.039)
$mm^3 \cdot 10^6$ particles^{-1}	0.286 (8; 0.021)	0.113 (8; 0.029)
% organics	81.101 (60; 0.431)	51.400 (25; 2.27)
mg organics·mm^{-3}	0.706	1.239
J·mg DW^{-1}	20.700 (8; 0.110)	9.909 (8; 0.214)
J·mg POM^{-1}	24.885 (8; 0.102)	18.401 (8; 0.263)
J·mm^{-3}	17.569	22.798
% total C·mg DW^{-1}	28.713 (8; 1.917)	22.287 (8; 1.356)
% organic C·mg DW^{-1}	23.290 (8; 2.104)	12.090 (8; 3.021)
organic C:N	5.0:1 (8; 0.021)	4.8:1 (8; 0.568)

important implications with respect to the volume of material the digestive tract is capable of holding (Bayne *et al.*, 1987).

A comparison between laboratory and surf-water particulates indicated that experimental particle counts, volume and DW l^{-1} were in the lower range of that found at Ouskip (Table 4.3). There was close agreement however, between organic DW and carbon values l^{-1}. Expressed as J l^{-1}, cultured algae presented a range which exceeded that found in the field. Organics per unit volume surf particulates were marginally lower (0.50 $\pm$ 0.18 mg organic DW mm^{-3}) than measured for laboratory food.

The similarity in C:N ratios between algae and detritus is a further indication of the latter's phytoplanktonic origin. In the laboratory, however, most of the sand was separated from seafoam and this could account for a ratio similar to algae but slightly lower than surf particulates, which naturally contains more refractory material.

Table 4.3. Comparison between quantity (range of estimates) and quality (mean estimates) of laboratory food and surf particulates from Ouskip. Data from other tables are included for ease of comparison.

	LABORATORY		SURF WATER
	T. suecica	detritus	
QUANTITY			
particle count $X\ 10^6 \cdot l^{-1}$	5.00- 50.00	5.00- 40.00	11.32- 82.45
$mm^3 \cdot l^{-1}$	1.47- 16.42	0.86- 4.42	1.07- 23.62
mg $DW \cdot l^{-1}$	1.25- 12.45	1.37- 10.92	2.22- 25.28
mg organic $DW \cdot l^{-1}$	1.01- 10.08	0.70- 5.61	1.06- 14.16
$J \cdot l^{-1}$	25.88-257.72	13.58-108.21	6.70-106.68
mg organic $C \cdot l^{-1}$	0.34- 3.36	0.31- 2.46	0.35- 4.05
QUALITY			
organic C:N	5.00	4.80	6.00- 9.00
% organics	81.10	51.40	42.66
$J \cdot mg\ DW^{-1}$	20.70	9.91	4.25
J·mg organic DW^{-1}	24.89	18.40	9.96
% organic C·mg DW^{-1}	23.39	12.09	6.68
mg organics·mm^{-3}	0.71	1.24	0.50
$J \cdot mm^{-3}$	17.57	22.79	4.99

CLEARANCE RATES

To calculate clearance rates it was assumed that particles >3 um were retained with 100% efficiency (see Matthews *et al.*, 1989) and that CR = pumping rate. A further assumption was that clearance of particles from suspension is continuous. Although *D. serra* was observed to filter continuously throughout the 8-hr experiments, rates could vary widely with time and diet, and between individuals of similar weight. Fig. 4.2 illustrates this and also shows that rates for bivalves fed algae varied more than when filtering detritus at similar densities (8.7 and 8.2 mg DW l^{-1} respectively).

It was notable that irrespective of food type or concentration, CR tended to accelerate in the last 2 to 3 hrs of each 8-hr experiment. An exception to this occurred when the highest algal ration was introduced (Fig. 4.3). At this concentration *D. serra* immediately increased filtration beyond previously measured rates and maintained this level for 3 to 5 hrs. Clearance eventually declined to very low rates or ceased altogether with siphons closed and valve-gape lessened. Final results are presented as means of the estimates made for each 20-min period thereby averaging out temporal variability in clearance rates.

Relationships between body size (mg DW) and clearance rates (l hr^{-1}) are given in Table 4.4 as allometric equations ($CR = a \cdot W^b$) and illustrated in Fig. 4.4. Regression slopes (*b*-values) indicate that CR increases with

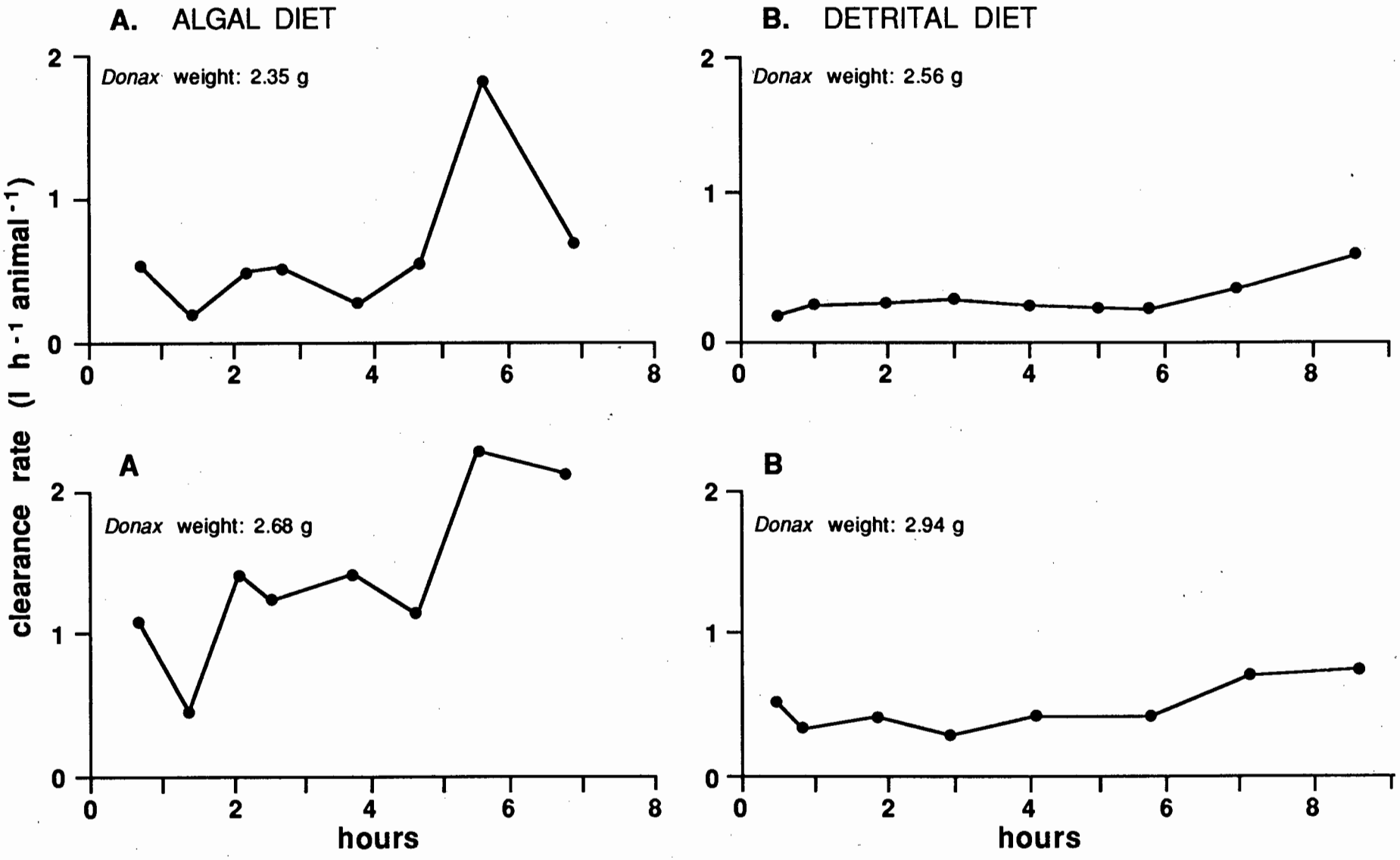

Fig. 4.2. Changes in clearance rate (l h $^{-1}$) over 8 hours in adult *Donax* of similar dry tissue weight fed (A) 35 X 10 ^{6} *T. suecica* cells l $^{-1}$ (8.72 mg DW l $^{-1}$) and (B) 30 X 10^{6} detrital particles l $^{-1}$ (8.19 mg DW l $^{-1}$) at 15 ^{o}C.

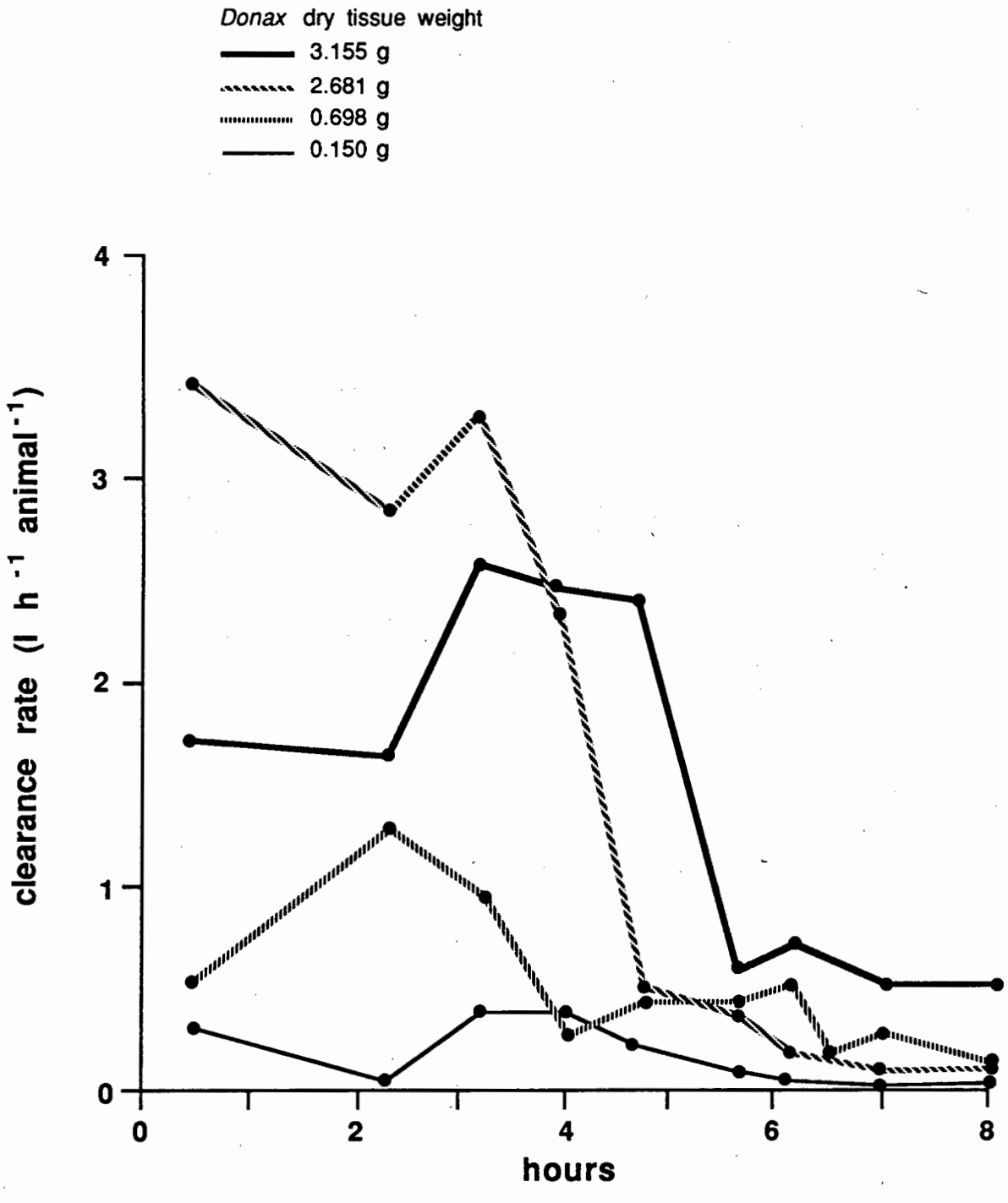

Fig. 4.3. Change in clearance rate (l h^{-1}) over 8 hours in *Donax* of different sizes fed 50 X 10^6 *T. suecica* cells l^{-1} (comparable to a ration of 12.45 mg DW l^{-1}) at 15 °C.

animal size, irrespective of diet or food concentration. The highly significant ($P > 0.005$) Pearson product-moment correlation coefficients (r) demonstrate the strength of this relationship.

The *a*-values (Table 4.4 and Fig. 4.4) indicate that clearance of *T. suecica* cells accelerated with increase in ration up to 25 X 10^6 cells l^{-1}, slowing down thereafter at 30 and 40 X 10^6 l^{-1}. The recurrent increase in CR at 50 X 10^6 cells l^{-1} shown in Fig. 4.4 was only temporary (<5 hrs) and was associated with the release of copious amounts of pseudofaeces.

Clearance of detritus steadily increased between 5 and 20 X 10^6 particles l^{-1}, followed by a decline with further increase in ration to 40 X 10^6 l^{-1} (Table 4.4) beyond which there was no recurrent rise in rates as measured with algae.

Analysis of covariance (ANOCOVA) showed no significant difference between *b*-values so that mean weight coefficients (b_c) equalled 0.529 for algae and 0.533 for detritus (Table 4.5). Thus food quantity had no effect on the manner in which different sized bivalves responded to increasing concentrations of algae or detritus. With no significant difference between diet-related *b*-values, it was possible to perform Newman-Keuls (NK) multiple range tests to find equality between elevations (*a*-values). There was no significant difference between rates at 5 and 40 X 10^6 l^{-1}, and 10 and 30 X 10^6 l^{-1}, that is between low and high

Table 4.4. *a*- and *b*-values from the allometric equation CR = $a \cdot W^b$ describing the relationships between clearance rate (CR in 1 hr^{-1}) and body size (W in g DW) of *D. serra* fed different rations of cultured algae (*T. suecica*) and detrital foam.

T. suecica					Detrital foam				
Cells X 10^6 l^{-1} [mg DW l^{-1}]	Clearance Rate 1 hr^{-1}				Particles X 10^6 l^{-1} [mg DW l^{-1}]	Clearance Rate 1 hr^{-1}			
	a	*b*	n	r		*a*	*b*	n	r
5 [1.25]	0.15	0.41	29	0.55	5 [1.37]	0.15	0.49	38	0.74
10 [2.49]	0.40	0.49	31	0.83	10 [2.73]	0.21	0.51	26	0.87
15 [3.74]	0.78	0.54	29	0.91					
25 [6.23]	1.05	0.55	22	0.93	20 [5.46]	0.31	0.62	44	0.90
30 [7.47]	0.99	0.49	19	0.94					
35 [8.72]	0.44	0.53	38	0.97	30 [8.19]	0.19	0.53	53	0.87
40 [9.96]	0.15	0.64	35	0.84	40[10.92]	0.16	0.54	29	0.89
50[12.45]	0.75	0.63	35	0.78					

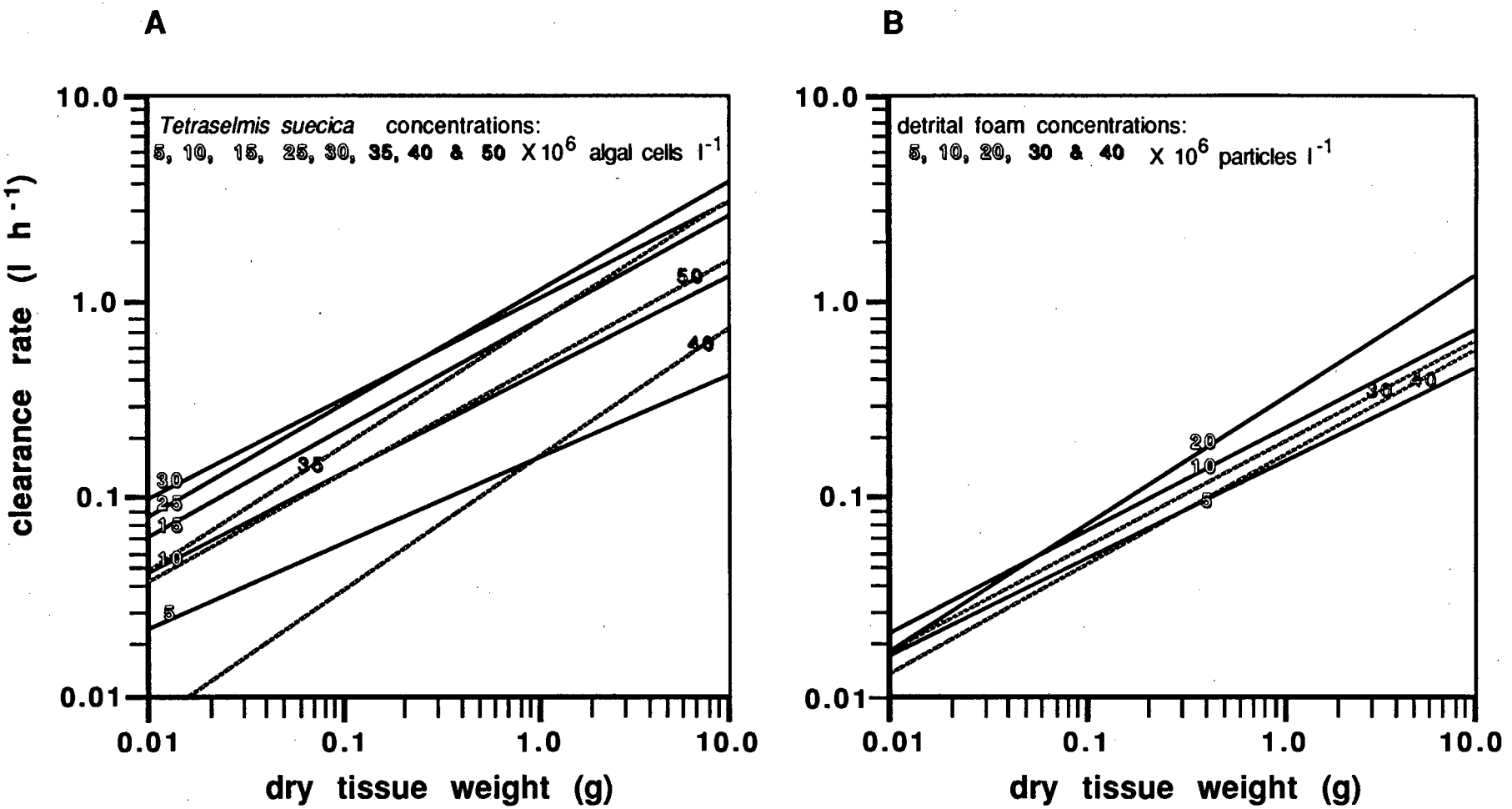

Fig. 4.4. Clearance rate (l h $^{-1}$) in relation to body size (g dry weight) on diets of *T. suecica* in the range 5 to 50 X 10 ^{6} cells l $^{-1}$ (A) and detrital foam from 5 to 40 X 10 ^{6} particles l $^{-1}$ (B) at 15 $^{\circ}$C. See Table 4.4 for equations for the lines.

rations, as well as between 15, 25 and 30 X $10^6 1^{-1}$, rations at which maximum clearance rates were reached (Table 4.5).

Although CR at 50 X 10^6 1^{-1} was included in this analysis, it is not a true reflection of ingestion. At this concentration, unlike at lower algal rations, most cells were rejected as pseudofaeces. Animals held at this algal concentration all showed distended digestive glands with stomachs and intestines packed with cells.

Multiple range testing on detrital data showed significant equality between clearance rates at 5, 10, 30 and 40 X 10^6 particles 1^{-1}, but clearance at 20 X 10^6 1^{-1} was greater than at any other ration level (Table 4.5). Maximum clearance of detritus thus occurred over a very restricted particle range.

Removal of detrital particles was substantially slower than for algae over a similar concentration range. For example, an increase in *T. suecica* from 1.3 (5 X 10^6) to 6.3 (25 X 10^6) mg 1^{-1} stimulated a 7-fold increase in CR (0.2 to 1.1 l hr^{-1} g^{-1}), whereas rates for nearly equal quantities of detritus (1.4 to 5.5 mg 1^{-1}) only doubled (from 0.2 to 0.3 l hr^{-1} g^{-1}). The significance of these differences was analysed using a Student-*t* test procedure in which regressions for algae and detritus of equivalent rations were compared (Table 4.5). No significant difference occurred between slopes. Elevations demonstrated significant similarity between rates at the lowest (1 mg 1^{-1}) and highest (10 mg 1^{-1}) rations. However, at equivalent

Table 4.5. Analysis of covariance and multiple range testing between clearance rates at different concentrations of the same type of food and between rates at equivalent quantities of *T. suecica* and detrital foam. Analysis procedure follows Zar (1982) using $\log_{10}$ transformed data.

ANALYSIS OF COVARIANCE $P < 0.01$

	T. suecica					Detrital foam				
Between	k	DF	F_s	F	b_c	k	DF	F_s	F	b_c
b-values	8	222	1.40	2.73	0.529	5	180	-16.44	3.41	0.533
a-values	8	236	40.25	2.73		5	184	12.52	3.41	

NEWMAN-KEULS MULTIPLE RANGE TEST
Only paired comparisons of elevations showing no significant difference are listed

Algae X 10^6 l^{-1}	q	p	$q_{0.01,222}$	Detri X10^6 l^{-1}	q	p	$q_{0.01,180}$
5 & 40	0.61	2	3.64	5 & 10	3.72	4	4.40
10 & 35	0.69	2	3.64	5 & 30	2.86	3	4.12
15 & 25	2.29	3	4.12	5 & 40	0.31	2	3.64
15 & 30	1.14	2	3.64	10 & 30	1.34	2	3.64
15 & 50	0.30	2	3.64	10 & 40	3.15	3	4.12
25 & 30	2.01	2	3.64	30 & 40	2.22	2	3.64
25 & 50	2.79	4	4.40				
30 & 50	2.28	3	4.40				
35 & 50	0.73	2	3.64				
Overall conclusion:				Overall conclusion:			
5=40, 10=35, 15=25=30=50, 35=50				5=10=30=40			

STUDENT-*t* TEST
Comparing regressions for algae and detritus of equivalent quantities

mg DW l^{-1}		Between slopes (*b*)			Betw elevations (*a*)		
Algae	detri	t_s	$t_{0.01(2)}$	DF	b_c	t_s	$t_{0.01(2)}$	DF
1.25	1.37	-0.50	±2.66	63	0.446	0.06	±2.66	64
2.49	2.73	-0.03	±2.68	53	0.505	4.66	±2.68	54
6.23	5.46	-0.99	±2.66	62	0.585	4.75	±2.66	63
8.72	8.19	0.26	±2.64	87	0.533	11.15	±2.64	88
9.96	10.92	1.10	±2.66	60	0.601	-0.69	±2.66	61

intermediate quantities, clearance of algal cells was significantly greater than for detritus.

INGESTION RATES

Allometric relationships between body size and ingestion rates are given in Table 4.6. These equations are illustrated as double logarithmic plots in Figs. 4.5 and 4.6 in terms of mg DW algae or detritus ingested hr^{-1} (IR) and weight-specific ingestion ($IR_{\%}$ = percentage body wt ingested day^{-1}) respectively. Since ingestion rates are directly influenced by clearance rates, *b* and r values in Table 4.6 describe similar relationships between ingestion and body weight (Table 4.4 & Fig. 4.4).

Maximum IR g^{-1}, reached at 30 X 10^6 algal cells l^{-1}, was 29% higher than at 5 X 10^6 l^{-1} (IR at 50 X 10^6 l^{-1} is not considered here since pseudofaeces were produced). Maximum ingestion of detritus also occurred around 30 X 10^6 l^{-1}, but was only 8% higher than that at 5 X 10^6 l^{-1}. The amount of material ingested (g DW hr^{-1}) is therefore a function of the quality of diet as well as quantity.

Daily amounts of particulates ingested expressed as a percentage of g dry body weight ($IR_{\%}$) in relation to concentration, decreased very rapidly with an increase in body size at all food concentrations (Fig. 4.6). A 0.1-g *D. serra* fed 30 X 10^6 *T. suecica* l^{-1} ingested 41% of its body weight compared to only 13% for one of 1 g and 6% for a 4-g individual.

Table 4.6. a- and *b*-values for the allometric equation $IR = a.W^b$ describing the relationships between ingestion rate (IR), expressed as mg DW hr^{-1} or % body weight ingested day^{-1}, and body size (W, g DW) of *D. serra* fed different rations of cultured algae (*T. suecica*) and detrital foam. Note: rate at *50 X 10^6 cells 1^{-1}* overestimates ingestion and is only included for completeness.

	Ingestion Rate mg DW hr^{-1}				Ingestion Rate % body wt. d^{-1}			
T. suecica X 10^6 l^{-1} [mg DW l^{-1}]	*a*	*b*	n	r	*a*	*b*	n	r
5 [1.25]	0.18	0.44	29	0.58	0.42	-0.57	29	-0.67
10 [2.49]	0.82	0.49	31	0.85	1.77	-0.55	30	-0.85
15 [3.74]	2.14	0.56	28	0.92	5.19	-0.44	29	-0.88
25 [6.23]	4.55	0.57	22	0.94	10.92	-0.43	22	-0.91
30 [7.47]	5.20	0.48	20	0.96	12.50	-0.52	20	-0.96
35 [8.72]	3.70	0.50	38	0.95	8.87	-0.50	38	-0.95
40 [9.96]	1.58	0.55	33	0.88	3.87	-0.47	35	-0.83
50[12.45]	*7.60*	*0.62*	*36*	*0.83*	*18.63*	*-0.43*	*35*	*-0.71*
Detritus X 10^6 l^{-1} [mg DW l^{-1}]	*a*	*b*	n	r	*a*	*b*	n	r
5 [1.37]	0.22	0.46	38	0.70	0.52	-0.56	38	-0.77
10 [2.73]	0.62	0.51	26	0.83	1.48	-0.49	26	-0.82
20 [5.46]	1.58	0.62	44	0.91	3.80	-0.38	44	-0.81
30 [8.19]	1.66	0.50	53	0.85	3.98	-0.50	53	-0.85
40[10.92]	1.37	0.53	29	0.90	3.05	-0.46	29	-0.82

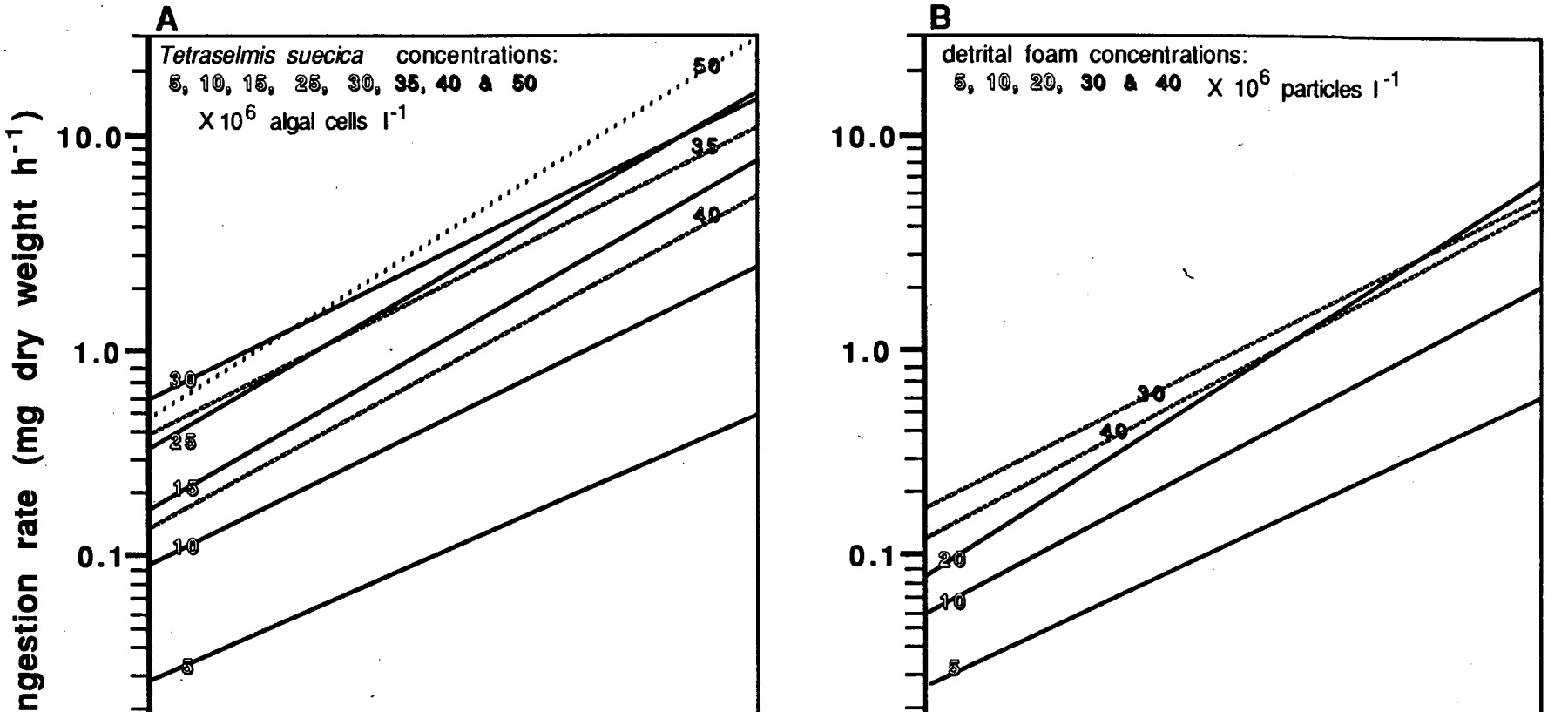

Fig. 4.5. Ingestion rate (mg dry weight h^{-1}) in relation to body size (g dry weight) on diets of *T. suecica* in the range 5 to 50 X 10^6 cells l^{-1} (A) and detrital foam from 5 to 40 X 10^6 particles l^{-1} (B) at 15 °C. See Table 4.6 for equations for the lines.

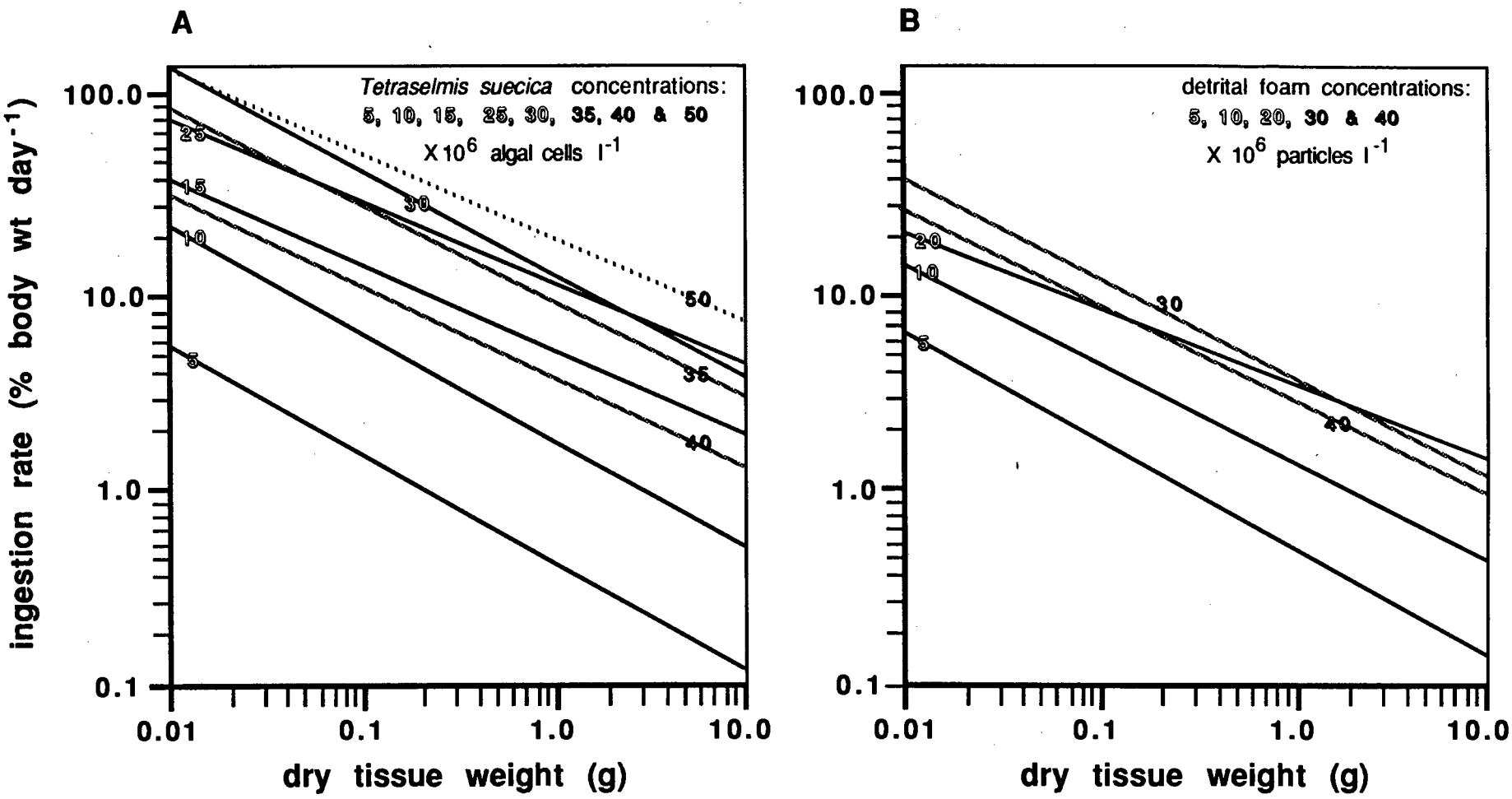

Fig. 4.6. Ingestion rate (% body weight day $^{-1}$) as a function of body size (g dry weight) on diets of *T. suecica* in the range 5 to 50 X 10^6 cells l^{-1} (A) and detrital foam from 5 to 40 X 10^6 particles l^{-1}. See Table 4.6 for equations for the lines.

Table 4.7. Analysis of covariance and multiple range testing between ingestion rates (mg DW hr^{-1}) at different concentrations of the same type of food and between rates at equivalent quantities of *T. suecica* and detrital foam. Analysis procedure follows Zar (1982) using $\log_{10}$ transformed data.

ANALYSIS OF COVARIANCE $P < 0.01$

	T. suecica					Detrital foam				
Between	k	DF	F_s	F	b_c	k	DF	F_s	F	b_c
b-values	8	221	0.81	2.73	0.523	5	180	1.72	3.41	0.544
a-values	8	235	128.89	2.05		5	184	63.78	3.41	

NEWMAN-KEULS MULTIPLE RANGE TEST
Only paired comparisons of elevations showing no significant difference are listed

Algae $X10^6$ l^{-1}	q	p	$q_{0.01,222}$	Detritus $X10^6$ l^{-1}	q	p	$q_{0.01,180}$
15 & 30	0.64	4	4.40	20 & 30	1.24	2	3.64
15 & 40	2.43	2	3.64	20 & 40	1.31	2	3.64
25 & 30	0.52	2	3.64	30 & 40	1.55	3	4.12
25 & 35	1.44	2	3.64				
30 & 35	0.56	3	4.12				
Overall conclusion: 15=30, 15=40, 25=30=35				Overall conclusion: 20=30=40			

STUDENT-*t* TEST
Comparing regressions for algae and detritus of equivalent quantities

mg DW l^{-1}		Between slopes (*b*)				Betw elevations (*a*)		
Algae	detri	t_s	$t_{0.01(2)}$	DF	b_c	t_s	$t_{0.01(2)}$	DF
1.25	1.37	-0.12	±2.66	63	0.446	-0.97	±2.66	64
2.49	2.73	-0.17	±2.68	53	0.498	2.03	±2.68	54
6.23	5.46	-1.55	±2.66	62	0.605	4.31	±2.66	63
8.72	8.19	-0.17	±2.64	87	0.503	10.26	±2.64	88
9.96	10.92	0.22	±2.66	58	0.542	1.33	±2.66	59

On a detrital diet of similar concentration, daily quantities ingested amounted to much smaller proportions of body weight. A 0.1-g bivalve ingested 13% of its body weight day^{-1}, a 1-g one, 4% and a 4-g individual, 2%. However, since a unit volume of seafoam detritus represents more organic material and energy than algae, it would not be necessary to fill the gut in order to achieve equivalent nutrient extraction.

Statistical analysis of similarities between ingestion rates (mg DW hr^{-1}) at different concentrations of either algae or detritus are shown in Table 4.7. No significant differences were found between slopes and thus common *b*-values (b_c) of 0.523 for algae and 0.544 for detritus were computed. For both sets of regressions, elevations were significantly different (ANOCOVA) and NK tests were again used to identify these differences. Ingestion rates were similar at the optimum algal rations (25 - 35 X 10^6 cells l^{-1}), and between low and high concentrations (15 & 30 and 15 & 40 X 10^6 l^{-1}). Since regressions for 15 and 40 X 10^6 l^{-1} showed similarity in part to those at 25, 30 and 35 X 10^6 l^{-1} (NK tests), any differences in ingestion rates over this entire range can be regarded as only weakly significant. Rates at 5 and 10 X 10^6 l^{-1} were significantly lower. On a seafoam diet, ingestion rates were similar at 20, 30 and 40 X 10^6 particles l^{-1}, but significantly less at 5 & 10 X 10^6 l^{-1}.

A comparison of regressions for algae and detritus of equivalent quantities showed no significant difference between slopes or between elevations at rations below about 6 mg and at 10 mg DW l^{-1} (Table 4.7). Ingestion rates at rations between 5 and 9 mg DW l^{-1} were however, significantly greater on an algal diet.

ABSORPTION EFFICIENCIES AND RATES

During experiments it was noted that the appearance of faeces and rate of faecal production differed in relation to food type and concentration. When ingesting algae in the range 5 - 10 X 10^6 cells l^{-1}, long thin strands (identified as algal faeces by the presence of lysed cells), appeared from the exhalent siphon 9 to 12 hrs after initial intake. Rations of between 15 and 35 X 10^6 cells l^{-1} resulted in predominantly long, thin faeces within 6 to 8 hrs, but short, thick strands, containing lysed and intact dead and live algal cells, were also produced. Short, thick strips, often filled with actively swimming *T. suecica*, were copiously excreted at densities >35 X 10^6 l^{-1} after 1 to 3 hrs. Pseudofaeces first appeared and continued to appear intermittently after prolonged exposure (>6 hrs) to 40 X 10^6 l^{-1}. Copious quantities emerged continuously after 30 to 60 mins at 50 X 10^6 l^{-1}.

These rates of faecal production provide an indirect measure of the residence times of algae in the gut. However, radioactive tracing of food through the digestive

tract would be the most accurate indication of residence times (see Stuart *et al.*, 1982b; Hawkins & Bayne, 1984).

Ingestion of foam detritus resulted in very long, thin faeces, identifiable by being dark brown compared to dark green, the colour of faeces excreted prior to and at the beginning of experiments. In the range 5 - 20 X 10^6 particles l^{-1}, these strands appeared after 14 to 18 hrs, and at 30 - 40 X 10^6 l^{-1}, after 8 to 10 hrs following initial ingestion. Unlike experiments with algae, quantities of detrital faeces did not escalate remarkably with an increase in particle density and pseudofaeces were only produced after long exposure (>6-8 hrs) to densities >40 X 10^6 particles l^{-1}. Although the heterogeneous nature of a detrital diet made it more difficult to assess gut residence times from content of faeces or rate of its production, it does seem that ingested detritus remained longer in the gut than *T. suecica*.

Absorption efficiencies (AE) for algae and detritus are compared in Fig. 4.7 in relation to food concentration expressed as particles l^{-1}, mg organics DW l^{-1} and Joules l^{-1}. Efficiencies for algae increased gradually from 37% at 5 X 10^6 to 71% at the optimum clearance and ingestion ration of 30 X 10^6 with a further small increase to 74% at 35 X 10^6 cells l^{-1}. Thereafter AE declined to 62% at 40 X 10^6 followed by a rapid fall to only 11% at 50 X 10^6 l^{-1}. Any absorption at this high cell density is probably only

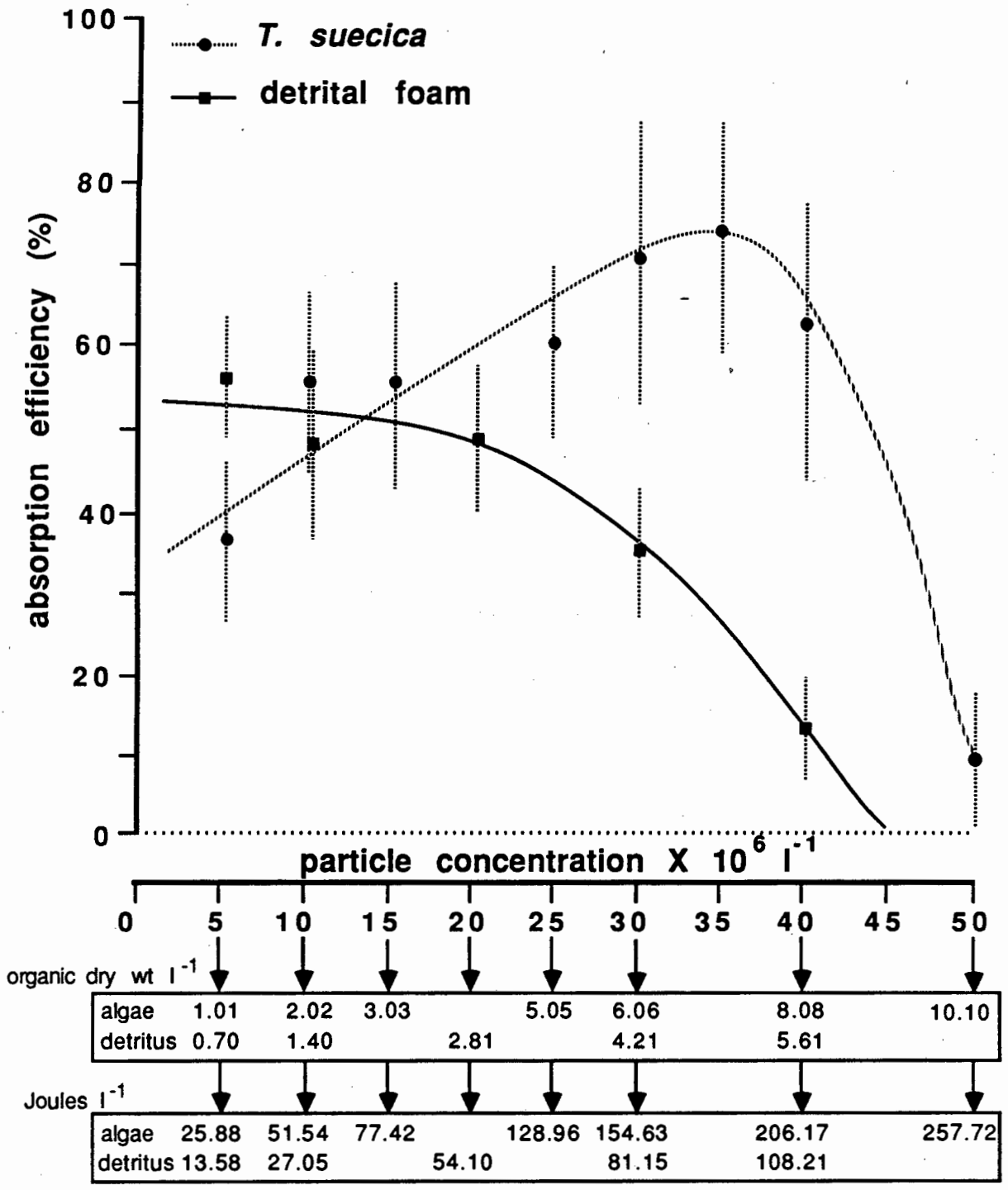

Fig. 4.7. Comparison between absorption efficiencies (%) at 15°C on diets of cultured algae and detrital foam over the particle range 5 to 50 X 10^6 l^{-1}. Vertical bars represent one standard deviation either side of the mean (n = 8).

incidental during the rapid shunting of ingested material through the digestive tract.

Efficiency of detrital absorption declined with an increase in ration. The highest efficiency was 57% at 5 X 10^6 l^{-1}, followed by a decline to 49% and 51% at 10 and 20 X 10^6 l^{-1} respectively. There was a further drop to 39% at 30 X 10^6 l^{-1}, a food concentration at which absorption of algae was increasing. At 40 X 10^6 particles l^{-1} AE was only 17%.

Differences in AE at similar quantitative and qualitative rations were analysed using Student-t tests ($t_{0.1;10}$). Efficiencies were only significantly different at particle concentrations >20 - 25 X 10^6 l^{-1}. At equivalent organic dry weights, AE only differed above 3 mg l^{-1} and in energetic terms, above 77 - 81 J l^{-1}.

Absorption efficiencies showed no dependency on body size, although values varied substantially between individuals of the same size on the same diet and at the same ration level. These inherent fluctuations are reflected in the wide standard deviations obtained for averaged data, especially on a diet of algae (Fig. 4.7).

Table 4.8 compares absorption efficiencies measured in laboratory experiments with those determined from the simultaneous collection of suspended particulates and faeces from surf at Ouskip. Absorption efficiencies recorded in the field were higher than those on equivalent food concentrations in the laboratory. These contrasts in AE probably relate to differences in food composition arising

Table 4.8. Comparison between total organics and organic carbon in faeces collected from surf at Ouskip and that excreted in the laboratory on diets of algae and detritus. Data chosen at equivalent dry weights l^{-1}.

	surf zone	*T. suecica*	detrital foam
FOOD SOURCE			
mg DW l^{-1}	5.3	5.9	5.5
% organics	49.0	81.1	51.4
C:N	7.0:1	5.0:1	4.8:1
FAECES			
% organics	17.8	59.9	34.2
C:N	8.5:1	6.0:1	5.4:1
ABSORPTION EFFICIENCY			
Percentage	79.7	64.1	50.1

from laboratory treatment of algae and seafoam detritus (i.e. drying, sieving and rinsing with ammonia formate). Disparity in feeding histories between experimental and field animals may also be a contributing factor.

The ratio of organic carbon to nitrogen was higher in faeces than food irrespective of diet (Table 4.8). This possibly indicates differential (and preferential) absorption of nitrogen rather than carbon or, excretion of material such as mucus that is metabolically derived within the alimentary canal.

Absorption rates, calculated as AR = IR X (% AE), are expressed as allometric equations in relation to body weight in Table 4.9 and illustrated as log/log regressions in Fig. 4.8 for the two laboratory diets. All animals maximised absorption of *T. suecica* at 30 X 10^6 l^{-1}, whereas on a detrital diet, rates peaked at 30 X 10^6 particles l^{-1} for individuals <0.2 g and at 20 X 10^6 l^{-1} for larger individuals (Fig. 4.8).

The magnitude of increase in the absorption rate of algae in response to increasing concentration far exceeded the same for detritus. For example, the rate at the optimum algal ration for a 1-g animal was 53 times that at the lowest concentration. By comparison, peak absorption of detritus amounted to only 6 times the rate at 5 X 10^6 l^{-1} (Table 4.9; Fig. 4.8). The magnitude of the decline in algal absorption at rations beyond the optimum was also greater than that of detritus.

Table 4.9. *a*- and *b*-values from the allometric equation AR = aW^b describing the relationships between absorption rate (AR, mg hr^{-1}) and body size (W, g DW) of *D. serra* fed different rations of cultured algae (*T. suecica*) and detrital foam. AR at *50 X 10^6 algal cells l^{-1}* is overestimated and only included for completeness.

T. suecica					Detrital foam				
Cells X 10^6 l^{-1} [mg DW l^{-1}]	Absorption Rate mg hr^{-1}				Particles X 10^6 l^{-1} [mg DW l^{-1}]	Absorption Rate mg hr^{-1}			
	a	*b*	n	r		*a*	*b*	n	r
5 [1.25]	0.07	0.45	29	0.60	5 [1.37]	0.13	0.46	38	0.70
10 [2.49]	0.45	0.50	31	0.85	10 [2.73]	0.30	0.51	26	0.83
15 [3.74]	1.19	0.57	28	0.92					
25 [6.23]	2.73	0.57	22	0.94	20 [5.46]	0.81	0.61	44	0.91
30 [7.47]	3.68	0.48	19	0.95					
35 [8.72]	2.74	0.50	38	0.95	30 [8.19]	0.65	0.50	53	0.85
40 [9.96]	0.98	0.55	33	0.88	40[10.92]	0.23	0.53	29	0.90
50[12.45]	*0.84*	*0.62*	*36*	*0.84*					

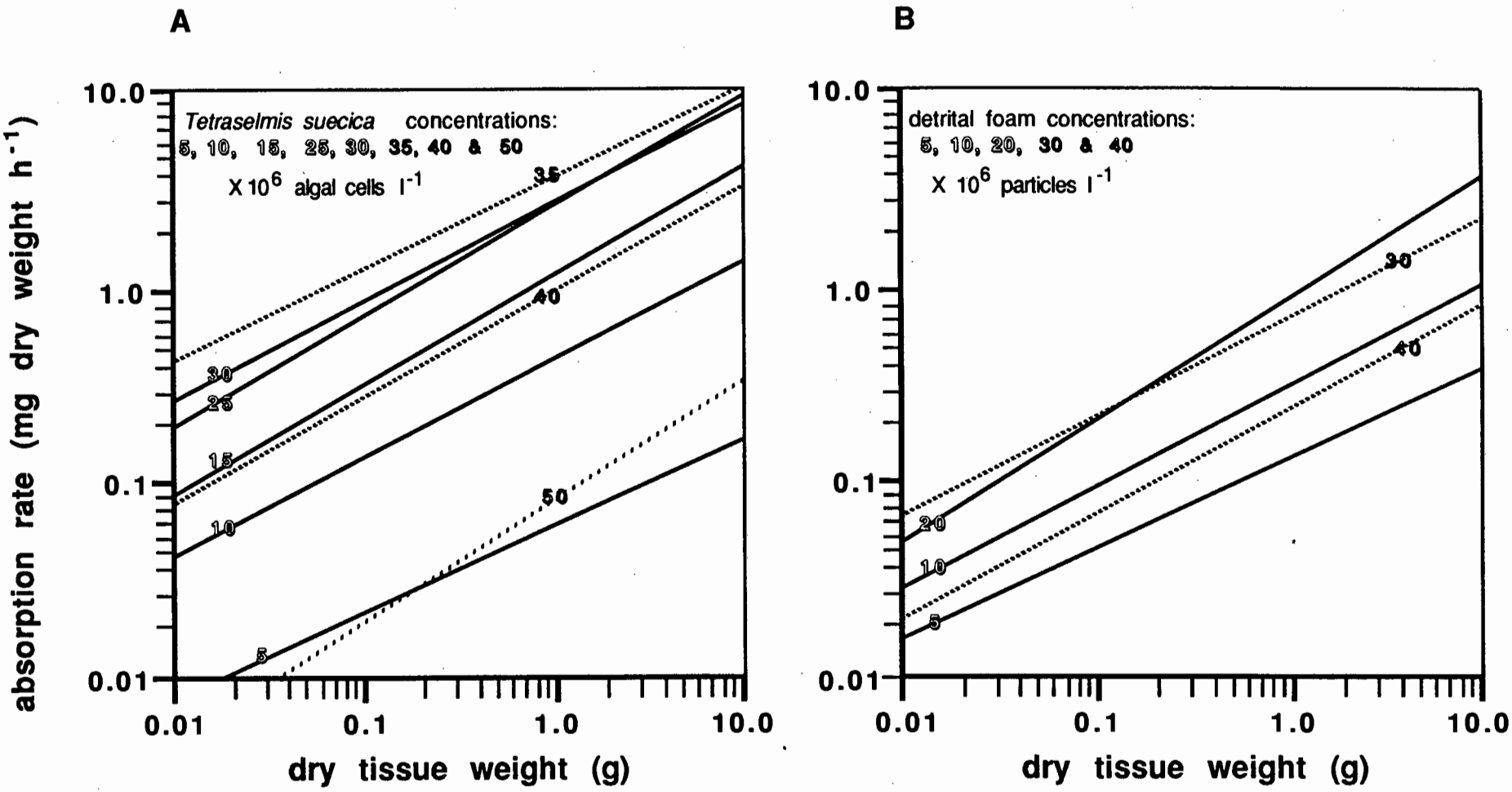

Fig. 4.8. Absorption rates (mg dry weight h^{-1}) in relation to body size (g dry weight) on diets of *T. suecica* in the range 5 to 50 X 10^6 cells l^{-1} (A) and detrital foam from 5 to 40 X 10^6 particles l^{-1} (B) at 15°C. See Table 4.9 for equations for the lines.

Covariance analysis and NK tests were again used to establish significant differences among absorption rates (Table 4.10). Size-rate relationships were not affected by either food quantity or quality. Maximum absorption rates demonstrated equality between 25 and 35 X 10^6 *T. suecica* cells l^{-1}. When feeding on seafoam detritus there was no difference between peak absorption measured at 20 and 30 X 10^6 particles l^{-1}.

Student-*t* analysis of regressions showed that absorption rates when feeding on *T. suecica* were significantly the highest above 3 mg l^{-1}, whereas below this ration level, absorption of detrital material was the greater. Thus the absorption rate of algae showed no significant equality to that of detritus at any equivalent ration level investigated between 1 and 11 mg DW l^{-1}.

Table 4.10. Analysis of covariance and multiple range testing between absorption rates (mg DW hr^{-1}) at different concentrations of the same type of food and between rates at equivalent quantities of *T. suecica* and detrital foam. Analysis procedure follows Zar (1982) using $\log_{10}$ transformed data.

ANALYSIS OF COVARIANCE $P < 0.01$

	T. suecica					Detrital foam				
Between	k	DF	F_s	F	b_c	k	DF	F_s	F	b_c
b-values	8	220	0.71	2.73	0.525	5	180	1.17	3.41	0.526
a-values	8	234	139.75	2.73		5	184	46.27	3.41	

NEWMAN-KEULS MULTIPLE RANGE TEST
Only paired comparisons of elevations showing no significant difference are listed

Algae $X10^6$ l^{-1}	q	p	$q_{0.01,220}$	Detritus $X10^6$ l^{-1}	q	p	$q_{0.01,180}$
15 & 40	1.92	3	4.12	10 & 40	2.02	2	3.64
25 & 30	2.68	3	4.12	20 & 30	1.90	2	3.64
25 & 35	0.26	2	3.64				
30 & 35	1.03	2	3.64				
Overall conclusion: 25=30=35, 15=40				Overall conclusion: 10=40, 20=30			

STUDENT-*t* TEST
Comparing regressions for algae and detritus of equivalent quantities

mg DW l^{-1}			Between slopes (*b*)			Betw elevations (*a*)		
Algae	detri	t_s	$t_{0.01(2)}$	DF	b_c	t_s	$t_{0.01(2)}$	DF
1.25	1.37	-0.06	±2.66	63	0.456	-2.95	±2.66	64
2.49	2.73	-0.14	±2.68	53	0.499	2.86	±2.68	54
6.23	5.46	-0.79	±2.66	62	0.595	12.10	±2.66	63
8.72	8.19	-0.02	±2.64	87	0.503	18.32	±2.64	88
9.96	10.92	0.23	±2.66	58	0.542	14.05	±2.66	59

RELATIONSHIPS BETWEEN CLEARANCE, INGESTION AND ABSORPTION RATES

Statistical analyses revealed a difference in the range of food concentrations over which maximum rates of clearance and ingestion were reached (NK tests in Tables 4.5 and 4.7 respectively). Clearance rates were maximal over the range 15 - 30 X 10^6 algal cells l^{-1}, whereas ingestion was greatest between 25 - 35 X 10^6 l^{-1}. Detrital clearance was maximal at 20 X 10^6 l^{-1}, whereas ingestion peaked over 20 - 40 X 10^6 l^{-1}. Irrespective of diet, ingestion was optimised at rations equal to or slightly greater than those at which the highest clearance rates were reached. As food concentrations increased further, clearance rates declined, but high ingestion rates were maintained. The contrast between fluctuations in CR and IR (expressed as J hr^{-1}) in response to increasing food quantities (mg DW l^{-1}) is more clearly demonstrated in Fig. 4.9 per 1 g DW.

The relationship between ingestion and absorption rates (Tables 4.7, 4.10, Fig. 4.9) highlights the influence of absorption efficiency on the quantity of food ultimately assimilated. Algal ingestion at rations from 15 - 40 X 10^6 l^{-1} were only marginally different (NK test, Table 4.7), but since efficiencies over this range reached their maximum as well as their minimum, absorption rates were significantly different. Algae ingested at the lowest and highest rations

were inefficiently absorbed, resulting in rates being significantly lower than at intermediate concentrations.

On a detrital diet, a 3-fold decline in absorption efficiency over 20 - 40 X 10^6 particles l^{-1}, resulted in a marked disparity between the quantity of food ingested and absorbed (Fig. 4.9b). As shown earlier in Table 4.7, absorption rate at the highest ration (40 X 10^6 l^{-1}) was significantly lower than at 20 or 30 X 10^6 l^{-1}.

The above relationships demonstrate a pattern of ingestion and absorption common to both diets, even though quantities of detritus consumed never matched algal intake. Ingestion and absorption increased with particulate concentration up to an optimal ration level beyond which ingestion remained (significantly) the same, whereas absorption rates declined. With both food types, the fall in AR at ration levels > 8 - 9 mg DW l^{-1} was a direct result of a dramatic decrease in absorption efficiencies and the production of pseudofaeces when feeding on algae.

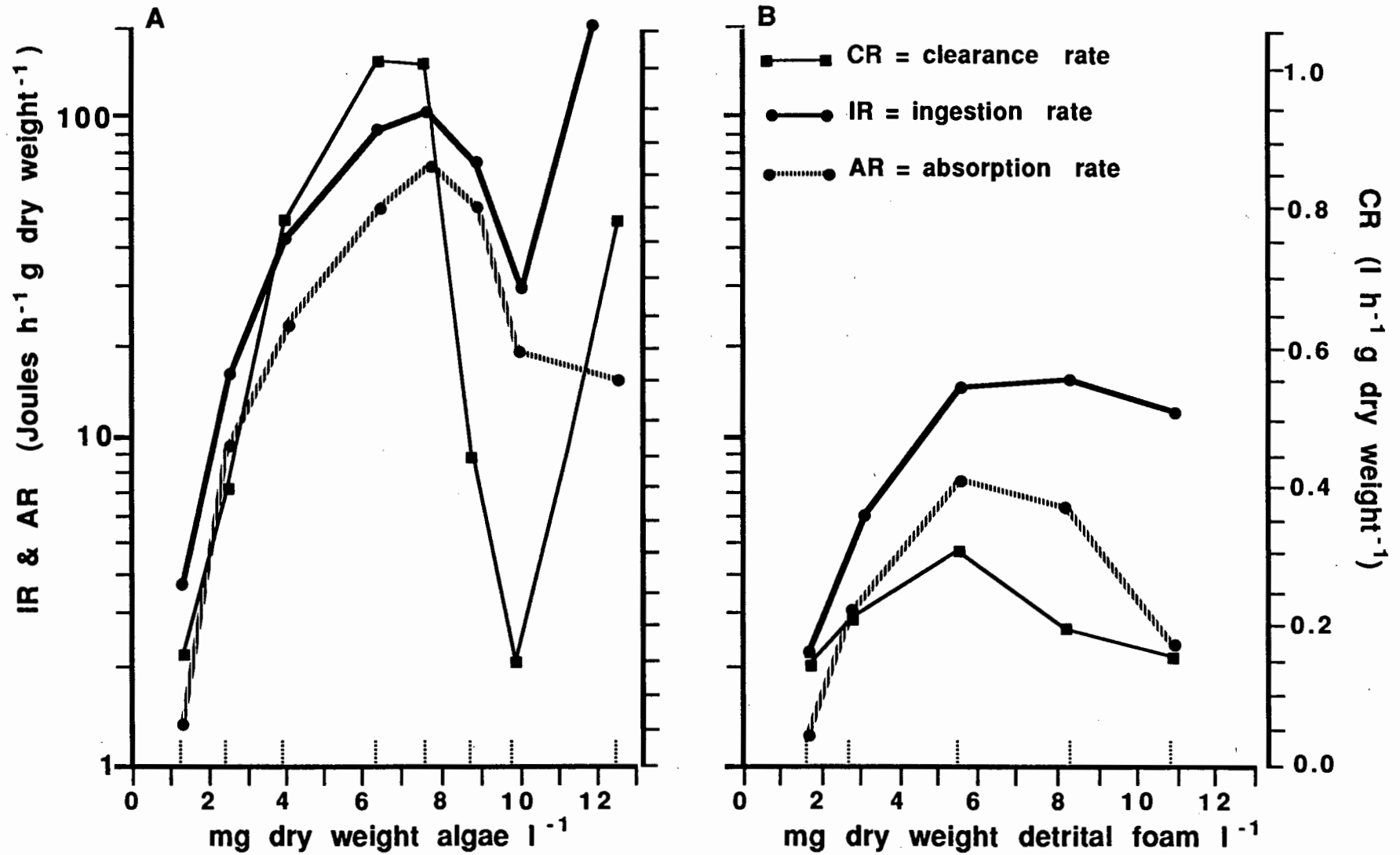

Fig. 4.9. Relationship at 15°C between clearance rate (CR = l $h^{-1}g^{-1}$) and energy ingested (IR) and absorbed (AR) in J $h^{-1}g^{-1}$dry tissue weight *D. serra* as a function of increasing rations (mg DW l^{-1}) of *T. suecica* (A) and detrital foam (B). These data are derived from the allometric equations in Tables 4.4, 4.6 and 4.7.
The dotted vertical bars indicate, progressively, the numerical density of algae and detritus used in experiments.

DISCUSSION

NATURAL FOOD AVAILABILITY

Availability of suspended material to *D. serra* and other filter feeders along the south-western coast of South Africa is strongly influenced by short-term cycles of upwelling and downwelling (Newell *et al.*, 1982; Newell & Field, 1983; Wulff & Field, 1983). During upwelling, south-east winds drive nearshore surface water offshore. This is then replaced by relatively plankton-free, nutrient-rich water from below the euphotic zone. Plankton blooms develop as this water moves seawards and is thermally stratified. When nutrients become limiting, the phytoplankton undergoes physiological and biochemical changes resulting in senescence and death and in the formation of particulate detritus (Barlow, 1982a). With downwelling, when north-west winds prevail, this detrital material, plus living plankton and other suspensoids, move inshore into kelp beds and surf zones (Andrews & Hutchings, 1980; Brown, 1981; Barlow, 1982b; Brown & Hutchings, 1987). Since south-east winds dominate in summer, strong upwelling occurs, on average, 42% of the time, medium upwelling, 25% and downwelling, the remaining 33%. In winter, calmer conditions or NW winds prevail 90% of the time, resulting in frequent downwelling (G. Nelson, quoted in Wulff & Field, 1983).

Monthly variations in quantity and quality of suspended material in surf water at Ouskip reflect the influence of

up- and down-welling. Particulates were always more concentrated and seafoam more prevalent when NW winds prevailed. This was especially evident in April, 1985 (autumn) when 25.28 mg DW and 10.75 ug chlorophyll *a* l^{-1} were measured. Average chlorophyll *a*, on the other hand, was slightly higher with SE winds, a surprising result seeing that such conditions are associated with newly upwelled water, relatively free of plankton and with negligible concentrations of chlorophyll *a* (Waldron, 1985). It has, however, been demonstrated that chlorophyll *a* in nearshore waters can be extremely variable in the short-term (Barlow, 1982b; Seiderer & Newell, 1985; Brown & Hutchings, 1987). A drop from 8.9 to 0.6 ug l^{-1} in 24 hrs was measured just south of Ouskip during a switch from a NW to SE wind by Seiderer & Newell (1985) . It is thus apparent that a mean taken from isolated measurements, as in the present study, cannot adequately reflect the association between chlorophyll concentrations and wind direction (or season).

The significance of chlorophyll *a* values is best expressed as a proportion of organic carbon. Such a ratio has been used to indicate the age and growth of phytoplankton blooms (Steele & Baird, 1962; Banse, 1977; Donaghay *et al.*, 1978; Andrews & Hutchings, 1980), as well as the proportion of carbon that originates from living phytoplankton (Seiderer & Newell, 1985; Newell & Langdon, 1986; Matthews *et al.*, 1989). In this context, the mean organic C :Chl *a* ratio of 72 recorded during SE winds at

Ouskip was suggestive of rapidly growing phytoplankton, whereas the much higher NW-mean ratio of 210 implied the predominance of decaying phytoplankton and other detrital material. Such particulate debris would have its origin beyond the breakwater. Bubble turbulence and flocculation in surface waters (Alldredge & Cox, 1982; Linley & Field, 1982; Prezelin & Alldredge, 1983) forms the aggregations observed to be more prevalent during onshore NW winds at Ouskip. This probably accounts for the greater proportion of larger sized particles encountered during north-westerlies.

Surf-zone particulate aggregations often reach a complexity and size (50 - 100 um) comparable to the `complex masses' described by Linley & Field (1982) from kelp beds. Due to intense turbulence in the surf however, such aggregations are highly unstable, disintegrating into smaller fragments, 99% of which were shown to be <20 um in diameter. *D. serra* retains this size fraction (>2 <20 um) with maximum efficiency (70 - 100%) (Matthews *et al.*, 1989). Nevertheless, although large aggregations >50 um are not an exploitable resource for *D. serra*, they do serve to concentrate and localise POM and DOM, as well as bacteria (Lucas, 1986).

The formation of seafoam via the vigorous agitation of microlayers of organic molecules is another important aggregation process (Velimirov, 1980; Barlocher *et al.*, 1988). At Ouskip, aggregations of foam on the leading edge

of waves yielded chlorophyll *a* measures as high as 508.60 ug l^{-1}. Ultimately foam was either deposited on the beach, or forced back into the water column by wave action where it was dispersed and resuspended to become food for *D. serra*. It was often noted that seafoam became trapped in swash currents which followed the course of troughs between cusps and horns, areas in which *D. serra* reach their greatest densities.

COMPARISON BETWEEN LABORATORY AND NATURAL FOOD

Any attempt to quantify the energy balance of an organism in its natural environment must involve a food source that best mimics natural particulate material. This is relatively easy in terms of dietary quantity, hence the extensive studies of the response of suspension-feeding bivalves to variations in food concentration reviewed by Winter (1978), Bayne & Newell (1983) and Griffiths & Griffiths (1987). Food quality has however, been largely neglected. In this study, natural particulate quantity was adequately reproduced in experiments, but quality was higher in laboratory cultured *T. suecica* and processed detrital foam. Nevertheless, of the two experimental foods, the more naturally-derived seafoam best mimicked surf particulates, not only in quality, but also in the complex composition of biogenic material originating from phytoplankton, detritus, bacteria and resuspended faeces.

The most commonly applied parameters of food quality have been organic and/or calorific content. Attempts to simulate natural particulates have usually entailed adding inert particles or silt derived from natural sediments to cultured algae (Winter, 1976; Kiorboe *et al.*, 1980, 1981; Bayne *et al.*, 1984; Bricelj & Malouf, 1984; Robinson *et al.*, 1984; Bayne *et al.*, 1987). Alternatively, the carbon and/or nitrogen content of food has been used to interpret trophic relations and resource transformations of bivalves (Kofoed, 1975; Kiorboe *et al.*, 1980; Seiderer *et al.*, 1982; Stuart *et al.*, 1982a, b; Hawkins & Bayne, 1985; Seiderer & Newell, 1985; Lucas *et al.*, 1987; Matthews *et al.*, 1989). Recent research has shown that ratios of carbohydrates, proteins and lipids in food can also be important quality criteria, especially with regard to growth (Flaak & Epifanio, 1978; Wikfors *et al.*, 1984; Mayasich & Smucker, 1986). Defining quality can be further complicated by the natural variability in the composition of a food resource. In a study on the growth rate of the deposit-feeding polychaete *Capitella capitata*, Tenore (1981; 1983) has shown that the relevance of nutritional quality based on calorific content on the one hand, and nitrogen on the other, depended on whether detritus was derived from seaweed or saltmarsh grasses.

The quality of natural particulates would also change with season and environmental conditions. The strongest evidence of seasonal change in suspensoid quality at Ouskip

was the significantly higher organic carbon to chlorophyll *a* ratios characteristic of downwelling and indicative of more detritus than phytoplankton during winter compared to summer. This did not coincide with any notable differences in other measures of quality such as organics, energy content and C:N ratios. This is consistent with the view that the C:N ratio may be a poor index of detrital nutritional value (Rice, 1982; Robinson *et al.*, 1982). Nevertheless, it is highly probable that biochemical composition, if assessed, would have shown strong seasonal trends since the food value of phytoplankton changes in relation to seasonal availability of nutrients (Barlow, 1982a; Wikfors *et al.*, 1984; Enright *et al.*, 1986). In the upwelling region inhabited by *D. serra*, carbohydrate content of living phytoplankton increases as upwelled water matures and the nitrate supply becomes depleted (Barlow, 1982a). A shift towards the dominance of detritus in this environment would also bring about biochemical variation since this material is higher in protein content than phytoplankton (Barlow, 1982a).

It is important, therefore, to rely on many measures of food quality and to take great care in the methods employed to mimic natural particulates. It is far more meaningful to use naturally-derived material (like seafoam in the present study) than either cultured algae or a mixture of silt and algae. Such an approach has proved relatively easy with deposit-feeding bivalves where natural food is readily

accessible as sedimentary detritus (Hylleberg & Gallucci, 1975; Wikander, 1981; Lopez & Cheng, 1982; Hummel, 1985). Natural suspended material is however, not as readily available and its use in filter-feeding studies is therefore less common. In experiments on the kelp-bed bivalve *A. ater*, powdered aged macrophyte fronds and resuspended mussel faeces were used as food (Stuart *et al.*, 1982a). In a similar manner, Lucas & Newell (1984) used powdered saltmarsh grasses when researching feeding in *Crassostrea virginica* and *Geukensia demissa*. A more recent approach has been to maintain bivalves during feeding experiments in sea water collected at the same time and from the same locality as the animals (Lucas *et al.*, 1987; Matthews *et al.*, 1989).

PARTICLE CLEARANCE

Temporal variability and nature of diet

The marked temporal variability in clearance rates, displayed by *D. serra* maintained with a constant concentration of *T. suecica* cells, is characteristic of suspension-feeding bivalves (Jorgensen, 1949; Winter, 1973; Schulte, 1975; Bayne *et al.*, 1977; Epifano & Ewart, 1977; Griffiths, 1980a; Palmer, 1980; Davenport & Woolington, 1982; Hawkins *et al.*, 1983; Bricelj & Malouf, 1984). It was initially presumed that such variability was associated with tidal cycles or season but this remains unsubstantiated (Jorgensen, 1960; Griffiths, 1980a; Griffiths & Buffenstein, 1981). The most likely explanation is a close link between

particle clearance/ingestion and endogenous rhythms of digestion, absorption and excretion as suggested by the work of Hawkins *et al.* (1983) on *Mytilus edulis*.

In view of the above it is worthwhile to recall the response of *D. serra to dense algal suspensions (50 X 106* cells l-1). Initially, clearance rates at this ration were high and variable, coincident with rapid ingestion in the first hour followed by the production of copious amounts of pseudofaeces and faeces containing intact algal cells. This indicates that once the digestive gland was full, cells were either still ingested, but shunted undigested through the alimentary tract and egested, or rejected as pseudofaeces. This implies a regulation between cell clearance and phases of ingestion, digestion and egestion. However, since this response was of short duration (3 to 5 hrs) with clearance rates eventually declining sharply, it is assumed that the ctenidia and digestive system became overloaded. In this respect, abundant secretion of mucus comprising pseudofaeces could also be viewed as a means of cleaning the gill surfaces, as suggested by Jorgensen (1976). Further indirect evidence of overloading was provided by the closure of siphons, as well as a reduction in shell-gape. Riisgard & Randlov (1981) also observed reduction of the opened position of the shell in M. edulis in response to high as well as very low algal densities.

In contrast to algae, seafoam particles were cleared at a lower rate, and showed very little temporal variability.

Even when supplied with surf water from the natural environment (Matthews *et al.*, 1989), *D. serra* displayed a weight-specific filtration rate less than when supplied algae. Such differences in rates relating to nature of diet have been recorded previously in marine bivalves (Hibbert, 1977; Mohlenberg & Riisgard, 1979; Bricelj & Malouf, 1984). Indeed, Doering & Oviatt (1986), comparing eight models of filtration rates, concluded that only those rates based on natural suspensions yielded good agreement with observed rates of particle depletion due to bivalves feeding in a mesocosm experiment. They found that models using dyes or algal monocultures overestimated gross depletion by up to an order of magnitude.

While clearance rates based on pure algal diets may be overestimated, it should be borne in mind that those using natural food may be underestimated since natural assemblages represent a much broader array of particles than do algal cultures. In studies on particle retention efficiencies, bivalves have been shown to clear larger particles preferentially (Stuart & Klumpp, 1984; Lucas *et al.*, 1987; Matthews *et al.*, 1989), leading to a progressive increase in the proportion of small particles and an apparent decline in filtration rates (Williams, 1982; de Villiers & Allanson, 1988).

The high silt content of seafoam (relative to silt-free algal cultures) probably played the most significant role in depressing clearance rates in *D. serra*. Some authors have

found that increasing the silt load in experiments can reduce clearance rates (Loosanoff & Tommers, 1948; Widdows *et al.*, 1979; Kiorboe *et al.*, 1980; Bricelj & Malouf, 1984), whilst others have noted no effect (Mathers, 1974; Rodhouse, 1978; Mohlenherg & Kiorboe, 1981). This discrepancy possibly arises from differences in the nature and concentration of the silt. Bivalves from environments with high inorganic seston concentrations usually display lower clearance rates than those from habitats with more organic material (Bayne *et al.*, 1984, 1987). Aged detritus relative to fresh debris has also been demonstrated to retard rates (Stuart, 1982).

During feeding experiments utilising algae, the siphons of *D. serra* were most often fully extended and open whereas with detrital foam, the length and aperture, especially of the inhalent siphons, were greatly reduced. Since this corresponds to high clearance rates with algae and depressed rates with detritus, postural changes in the siphons may play a role in controlling pumping. A similar control has been observed in *M. edulis* by convergence of the mantle-edge tentacles across the inhalent opening and in other bivalves by closure of the exhalent siphon (Foster-Smith, 1976; Bayne & Newell, 1983).

It is possible that postural changes in *D. serra*'s siphons arise from a chemosensory response to food quality via the numerous ciliated sensory receptors on the inner and outer wall of the siphons and especially at the tip of the

tentacles encircling the inhalent aperture (Hodgson & Fielden, 1984). This comprises six primary, pinnately-branched tentacles interspersed with smaller secondary and tertiary ones, the whole forming a fine-meshed sieve across the opening (Ansell, 1981). A reduction in aperture diameter would result in closer meshing of the tentacles and a more refined "sieve" which may play some part in pre-clearance selection other than preventing sand grains from entering the mantle cavity. Adduction of the valves brings about some backward propulsion of water which was seen to eject sand (and possibly smaller particles) from the surface of the inhalent tentacles. Such rejection could contribute towards the observed reduction in clearance rate of seafoam particles as well as the lack of temporal variability in that rate. However, far more research is required to substantiate this postulated function of the siphons and their ciliated receptor sites which have also been observed in the bivalves *Donax sordidus*, *Solen capensis* (Hodgson & Fielden, 1984) and *Lima* (Owen & McCrae, 1979). It is generally accepted at the moment that particles are indiscriminately cleared from suspension and that the first sites of selection are the gills and labial palps (Hylleberg & Gallucci, 1975; Kiorboe *et al.*, 1980, 1981; Mohlenberg & Kiorboe, 1981; Newell & Jordan, 1983; Peirson, 1983; Shumway *et al.*, 1985).

Body size

The most useful aspect of research into the relation between body size and clearance rates lies in establishing a value for *b* in the allometric equation, $CR = a \cdot W^b$, which then allows extrapolation throughout the population size range by substituting for dry tissue weight (W). In this study, *b*-values also indicated that food quantity and quality had no effect on the manner in which different sized *D. serra* responded to increasing concentrations of algae or detritus.

Weight exponents among bivalves show considerable variation over the range 0.4 to 0.8 (Griffiths & Griffiths, 1987), with a mean value of 0.62 calculated by Bayne & Newell (1983). This approximates the theoretical relationship between mass and surface area (surface area = $mass^{0.67}$). The *b* values for *D. serra* were lower than this, showing significant parity at 0.529 on an algal diet and 0.533 with foam detritus. Weight exponents from studies using larger-sized bivalves like *D. serra* often tend to be lower than those only incorporating juveniles (Bayne, 1976). Although large bivalves do filter more water, a relative reduction in pumping rate per unit gill area with increase in body size (Vahl, 1973a) would account for a gentler slope to the regression of tissue weight against clearance rate in large species (Bayne, 1976).

Particle concentration

It is of interest to consider the relationship between clearance rate and concentrations of algae and detritus in

the context of the three-phase response postulated to be widespread among bivalves by Winter (1977) and supported by subsequent research (Widdows, 1978; Griffiths & King, 1979a; Riisgard & Mohlenberg, 1979; Widdows *et al.*, 1979; Griffiths, 1980a; Navarro & Winter, 1982; Wilson, 1983; Bricelj & Malouf, 1984). Briefly, this entails an initial rapid increase in clearance rate initiated by a particle concentration which exceeds some low threshold level, followed by a steady rate over a ration range presumably optimal for feeding, and finally, a progressive decline in clearance rate often accompanied by the production of pseudofaeces. The feeding behaviour of *D. serra* was generally consistent with Winter's conceptual scheme except for the recurrent short-term increase in clearance rate at high concentrations of algae (50 X 106 cells 1-1).

Foster-Smith (1975) identified two different strategies in bivalves to control ingestion at high food concentrations. There was a reduction in clearance rates as illustrated by *Arctica islandica* (Winter, 1970), *Cardium edule* and *Mercenaria mercenaria* (Bricelj & Malouf, 1984) and/or an increase in pseudofaeces production as in *Crassostrea virginica, M. edulis* and *Spisula subtruncata* (Haven & Morales-Alamo, 1966; Kiorboe *et al.*, 1980; Mohlenberg & Kiorboe, 1981). *D. serra* demonstrated both strategies and in addition, an ability to enhance particle rejection by rapid egestion of faeces containing an abundance of undigested material.

Bricelj & Malouf (1984) postulated that high selection efficiency and abundant pseudofaeces production indicated an advanced ability to cope with dense sediment suspensions and was therefore characteristic of species (like *D. serra*) which inhabit and exploit turbid environments. Nonetheless, it seems incongruous that liberal pseudofaeces release by *D. serra* should be a feature of an unnatural mono-algal diet rather than of detrital foam containing sediment. It is most likely therefore that at Ouskip, especially during winter when suspended material concentrates in the surf zone, *D. serra* controls ingestion primarily by reducing clearance rates.

It is noteworthy that on a diet of algae compared to detritus, clearance rates were not only greater but also peaked over a much wider range of food concentrations. Even though a diet of monocultured algae is recognised as artificial, this contrast points to an adaptive ability which could very well operate in response to peaks in phytoplankton blooms as well as to their periodicity, duration, density and food value. That is, *D. serra* could be seen as behaving opportunistically in its natural environment by maximising particle clearance at times of both moderate and abundant supply of high quality food. This would be a significant adaptation toward control over nutrient gain.

INGESTION, ABSORPTION AND EGESTION

Body size

Allometry among bivalves has shown that daily amounts of food ingested as percentage of dry tissue weight are greater in small individuals (see Bayne, 1976; Bayne & Newell, 1983; Griffiths & Griffiths, 1987). Data from this study were in agreement and demonstrated further that dietary quality strongly influenced weight-specific ingestion rates. At optimum food concentrations, percentage ingestion for a specific sized individual was 3 times greater when fed algae compared to detrital foam, this difference relating to the effect of quality on clearance rates. Since juvenile *D. serra* inhabit the mid-intertidal, they are covered by the tide for 3 to 6 hrs every tidal cycle. High weight-specific ingestion during submergence would thus be advantageous for positive growth.

Size-specific changes in ingestion rates might be expected to influence absorption efficiencies (Griffiths & Griffiths, 1987). However, like *D. serra*, efficiency is independent of body size in such species as *Modiolus modiolus* (Winter, 1969), *M. edulis* (Vahl, 1973b; Thompson & Bayne, 1974), *A. ater* (Griffiths & King, 1979a; Stuart *et al.*, 1982b), *Choromytilus meridionalis* (Griffiths 1980b) and *Mytilus chilensis* (Navarro & Winter, 1982). By contrast other studies have shown that absorption efficiency can either increase or decrease with size (see Griffiths & Griffiths, 1987). These results are not necessarily

contradictory, but rather reflect natural variability in efficiencies which obscures a clear relation to body size. Such variability was apparent in the high standard deviations of averaged absorption efficiencies for all sizes of *D. serra*, irrespective of dietary quality or quantity.

Application of the ash-ratio method (Conover, 1966) in itself generates variability in absorption efficiencies by ignoring organics in faeces that are derived metabolically in the gut rather than from the food. According to Famme & Kofoed (1982), a greater proportion of organic carbon in faeces relative to food indicates the release of such alimentary metabolites. This was true for *D. serra* with diets of algae, foam detritus and surf particulates.

Isotopic tracing of organics has shown that absorption efficiencies can be 30% higher than indicated by the Conover ratio, this being the difference between gross and net efficiencies (Hawkins & Bayne, 1984). Of more importance however, is that much of the fluctuation observed in efficiencies mirrors variable coupling between absorbance and the component activities of clearance, ingestion and digestion (Purchon, 1971; Morton, 1973; Hawkins *et al.*, 1983; Hawkins & Bayne, 1985).

Food concentration

The relationship between food concentration and ingestion rate in *D. serra* closely paralleled that for clearance rates until the pseudofaeces production threshold was reached. Rejection of food at the gill surface and/or labial palps to

form pseudofaeces demonstrated the capacity of the alimentary tract and digestive gland had been exceeded (see Jorgensen, 1981, 1983; reviews by Bayne & Newell, 1983; Griffiths & Griffiths, 1987). A drop in absorption efficiencies to below 20% at high food levels, coincidental with the faster egestion of faeces containing an abundance of undigested material, were further evidence of an overloaded digestive system in *D. serra* (see Van Weel, 1961; Thompson & Bayne, 1972; Riisgard & Mohlenberg, 1979).

The threshold concentration for pseudofaeces production by *D. serra* was high (9 - 12 mg DW l^{-1}) compared to that for other species with most values reported in the literature being between 1-6 mg DW l^{-1} (Bayne & Newell, 1983; Griffiths & Griffiths, 1987). However, included here are examples of species such as *Crassostrea virginica*, *M. edulis* and the surf clam *Spisula solidissima* (Robinson *et al.*, 1984), which release pseudofaeces regardless of food concentration, especially if silt is part of the diet. This response is indicative of pre-ingestive selection whereby nutritionally poor particles are rejected as pseudofaeces resulting in an increase in the food value of the ingested ration (Jorgensen, 1966; Kiorboe & Mohlenberg, 1981; Mohlenberg & Kiorboe, 1981; Newell & Jordan, 1983). In such species there is no longer a direct relationship between clearance and ingestion rates.

Since *D. serra* did not produce pseudofaeces at low to moderate concentrations of algae or detrital foam, pre-

ingestive selection did not appear to play a role in controlling food assimilation. Control was rather achieved by increased consumption over an extended food concentration range (approximately 5-9 mg DW l^{-1}) coincidental with maximum absorption efficiencies.

Casual observations on the egestion rate of faeces suggest that at low food densities, slower gut passage times may result in elevated absorption efficiencies. Similarly, Bayne *et al.* (1987), using isotope tracer techniques, found that in *M. edulis*, increased gut content and slower gut passage time were associated with reduced food availability.

Food quality

Influence of dietary quality on ingestion rates of *D. serra* was only apparent at rations between 5 and 9 mg DW l^{-1}, when the consumption of algae was significantly faster than that of detritus. Similarly, Stuart (1982) found that *A. ater* ingested kelp detritus at a slower rate than *Dunaliella* cells but only at rations above approximately 2 mg DW l^{-1}. This supports the theoretical model of Taghon (1981), based on studies of deposit feeders, which predicts that the most energetically optimal behaviour coincides with an increase in feeding rate in response to an increase in food quality. However, it is clear that such behaviour would, in addition, be dependent on the concentration of available particulates.

Experiments in which algal food sources were impoverished by adding silt have shown that increasing inorganics in the diet depressed ingestion rate in some

species (Winter, 1970, 1976; Foster-Smith, 1975; Bricelj & Malouf, 1984; Robinson *et al.*, 1984). In others, ingestion was unaffected or increased slightly (Haven & Morales-Alamo, 1966; Kiorboe *et al.*, 1980; Mohlenberg & Kiorboe, 1981; Bayne *et al.*, 1987). This apparent antithesis highlights the complexity of attempting to correlate ingestion rate directly with food quality. Factors such as the efficiency of pre-ingestive selection and discontinuous feeding in response to invariant food supplies, make it difficult to define a generalised and energetically optimal relationship between ingestion rate and food quality.

In this study interpretation of the effects of food value was further complicated by the fact that detrital quality proved marginally superior when expressed as mg organic DW per unit volume of particles. Foam detritus covered a much broader particle spectrum than *T. suecica* cells, resulting in a more densely packed unit volume of particles. Although this would have no bearing on the rate of ingestion, there are important post-ingestive implications, since a greater organic content can be achieved with detritus as food (assuming of course that a crammed gut is the optimal condition). Indeed, Bayne *et al.* (1987) found that when *M. edulis* was fed mixed algal-silt suspensions, quality expressed in this manner correlated far better with absorption efficiency than per unit dry weight of seston, a feature not apparent in the present study.

Whatever the manner of expressing quality, most research has shown that suspension-feeding bivalves generally absorb natural seston with efficiencies that are lower than for living algal diets (Bayne & Newell, 1983). This is true for *D. serra* when fed *T. suecica* and detrital foam. However, absorption efficiency estimated from food and faeces collected in the field was higher than at equivalent algal and detrital concentrations, namely 80% compared to 64% and 50% respectively. This may reflect the finding by Bayne *et al.* (1987) that extended exposure to a consistent food regime (i.e. longer than the 8-hr duration of laboratory experiments) can elevate absorption efficiency.

At low ration levels (less than 3 - 4 mg DW l^{-1}) absorption efficiencies were higher when *D. serra* was feeding on foam detritus instead of algae. This may arise because of the longer gut passage time of detritus (Bayne & Newell, 1983; Bayne *et al.*, 1987) and due to effective post-ingestive sorting, whereby nutritionally rich particles are differentially digested leaving the more refractory material to be egested (Bayne & Newell, 1983; Bricelj & Malouf, 1984; Cucci *et al.*, 1985; Shumway *et al.*, 1985). Naturally such a mechanism would become obsolete on a uniform diet like *T. suecica*, as well as on a heterogeneous detrital diet once the digestive gland was full and increased amounts of organics appeared in faeces, leading to a decline in absorption efficiency.

Although this study was not designed to elucidate the processes of either pre- or post-ingestive selection, circumstantial evidence does allude to the latter mechanism being the more important in the digestive physiology of *D. serra*. With the advent of new techniques in flow-cell cytometry (Yentsch *et al.*, 1983; Cucci *et al.*, 1985); research into post-ingestive selection promises to provide new insight into the processes governing nutrient acquisition in bivalves.

CONCLUSIONS

1.) The availability of surf particles to *D. serra* is dependent on episodic up- and down-welling events which in themselves show a seasonal trend, so that the most concentrated suspensions occur in winter. Surf turbulence results in the formation of particulate aggregations, most of which are in a size range available to *D. serra*.

2.) The best measure of surf-particulate quality was organic carbon to chlorophyll *a* ratios rather than energy content, organics or C:N ratios. Detrital material was shown to dominate in winter whereas phytoplankton suspensions prevailed in summer.

3.) Detrital foam, with its natural biogenic composition, was a better reproduction of surf particulates than algae cultured in the laboratory. Natural suspensoids are however, best reproduced in the laboratory by maintaining

bivalves in unfiltered sea water collected from their own environment.

4.) Temporal variation in clearance rates is a function of food quality and is more pronounced on a richer diet such as algae. It is probable that such variation reflects endogenous rhythms of digestion, absorption and excretion.

5.) Changes in the length and aperture width of the inhalent siphon are postulated to play some part in controlling clearance and ingestion rates. It is possible that postural changes in siphons are brought about by the response of sensory receptors to food quality. This would be consistent with the observed decline in clearance rates in the presence of detrital particles inferior in quality to algae.

6.) Ingestion was maximised in the short-term by increasing clearance rates over an optimal food range concomitant with temporal maxima in absorption efficiency.

7.) Pre-ingestive selection was not an apparent means of regulating the quality of the ingested ration, making it more likely that post-ingestive sorting determined ultimate nutrient acquisition.

8.) Food quality affected the manner in which ingestion was regulated at high particulate densities. Algae-fed *D. serra* displayed three distinct strategies: a) reduced clearance rates and increased pseudofaeces production; b) increased clearance accompanied by copious release of pseudofaeces and faeces containing an abundance of undigested material; c)

cessation of particle clearance by closing the siphons and partially closing the valves. Detritus-fed animals reduced clearance rates together with a small release of pseudofaeces.

9.) Absorption efficiency determined in the laboratory on diets of both algae and foam detritus underestimated the efficiency with which surf particulates were absorbed by *D. serra* at Ouskip. This highlights the difficulty of extrapolation, even when using naturally-derived particulate material.

10.) *D. serra* is an opportunistic filter-feeder. This species has demonstrated an adaptive response to food quantity and quality in the short-term by efficiently ingesting and absorbing large quantities of high quality food when it is optimally available. Since high quality food in this study was represented by cultured algae, it is postulated that opportunistic feeding would be advantageous to growth and reproduction during peaks in phytoplankton abundance in the surf zone at Ouskip.

CHAPTER FIVE

RATES OF OXYGEN CONSUMPTION AND AMMONIA EXCRETION

INTRODUCTION

The previous chapter was concerned with the nutrition acquired from the absorbed ration (Ab = IR X AE) without regard for the ways in which this was expended metabolically. In studies on the physiological energetics of bivalves, two main sources of energy loss have been identified. These are those due to heat production during the oxidation of carbohydrate and lipid substrates (R) and those due to excretion (U), which is primarily the loss of end-products of protein metabolism. In most research into the balance between energy gain and loss, R and U have been measured as calorific equivalents of rates of oxygen consumption and ammonia-nitrogen excretion respectively. This results in the familiar expression, P = C - (R + U + F) = Ab - (R + U), where P = SFG = scope for growth and reproduction, C = energy in the food ingested and F = egestion. SFG provides a physiological measure of net change in energy content of the body over time (Winberg, 1956; Warren & Davies, 1967; Bayne, 1976; Bayne & Newell, 1983).

The purpose of this chapter is to determine the rates of oxygen consumption and ammonia-N excretion in *D. serra* so that energy budgets can be constructed (Chapter 6). R and U however, both vary in relation to a number of intrinsic and extrinsic factors. Oxygen uptake may be affected by such factors as body size and level of activity, as well as by changes in virtually any environmental parameter (e.g.

temperature, salinity, O_2 concentration, ration level) [Widdows, 1978a; Shumway, 1983; Bayne & Newell, 1983; Bayne *et al.*, 1987]. As far as excretion is concerned, there is evidence of altered rates during starvation, in relation to differences in body size, reproductive condition and cyclic digestive patterns (Hammen, 1968; Emersen, 1969; Bayne, 1973; Ansell & Sivadas, 1973; Bayne *et al.*, 1976; Bayne & Scullard, 1977a; Hawkins *et al.*, 1985; Bayne *et al.*, 1987).

This chapter considers the scope for variability by establishing predictive equations relating R and U to the size of bivalves which have been starved, fed or are in the process of filtering suspended material. The nutritional status of the animal is used to define standard and routine metabolism, the difference between the two activity levels being assumed to represent the cost of particle clearance, digestion and assimilation. In keeping with the previous chapter, in which rates of food acquisition were related to dietary quality, animals were supplied with cultured algae and seafoam detritus. The effects of ration level are also considered, while effects of temperature and chlorine are confined to the concluding chapter of this thesis.

MATERIAL AND METHODS

MAINTENANCE OF ANIMALS

A representative size range of *D. serra*, collected from Ouskip beach between October and December 1986, were maintained at 15°C in flow-through aquaria fitted with air-lift pumps and provided with sand for burrowing. Animals were retained for one week before starting experiments and during this time were fed a mixed diet of *Tetraselmis suecica, Tetraselmis chuii, Dunaliella primolecta* and seafoam detritus.

Respiration and excretion experiments were conducted at 15°C on animals that were starved, fed and in the process of feeding. Starvation was induced by holding animals in 0.45-um filtered sea water for two weeks, whilst a well-fed group was established by supplying a mixture of *T. suecica* and seafoam detritus every day over the same time period. The third group were fed in the holding tanks as well as during experiments when rations of 10, 20 and 30 X 10^6 *T. suecica* cells l^{-1} and detritus in the range 20-30 X 10^6 particles l^{-1} were provided. These food concentrations were chosen as they stimulate maximum clearance and ingestion with respect to each food type (see Chapter 4). At all times sand, which had been thoroughly rinsed in 0.45-um filtered sea water, was provided for burrowing.

Respiration rate in starved individuals was taken to represent standard metabolism and that in fed and feeding *D. serra* to denote two distinctive levels of routine metabolism (see Navarro & Winter, 1982; Bayne & Newell, 1983). It is considered that active metabolism would only be represented by burrowing and surfing, activities which were not considered in this study.

OXYGEN CONSUMPTION EXPERIMENTS

A flow-through respirometer, fitted with YSI (Yellow Springs Instrument Co.) dissolved-oxygen probes and housed in a constant temperature room set at 15°C, was used to measure rates of aerobic respiration (Fig. 5.1). This system consisted of an upper reservoir from which sea water at 15°C flowed via four respiration chambers into a bottom reservoir from which sea water was pumped back up to the top reservoir. The chambers, fitted with YSI probes, could be instantly shut off from flowing sea water by tourniquet valves on the inlet and outlet hoses, or by closing the exit-taps attached to the top reservoir. The chambers were submerged in a tank of sea water to prevent air-leakage and magnetic stirrers below the tank ensured circulation within them. The probes were linked to an oxygen meter interfaced to an Apple II computer.

Within each respiration chamber, a bivalve was placed in a small glass container resting on a perforated disc and was filled with clean sterilised sand. In the case of

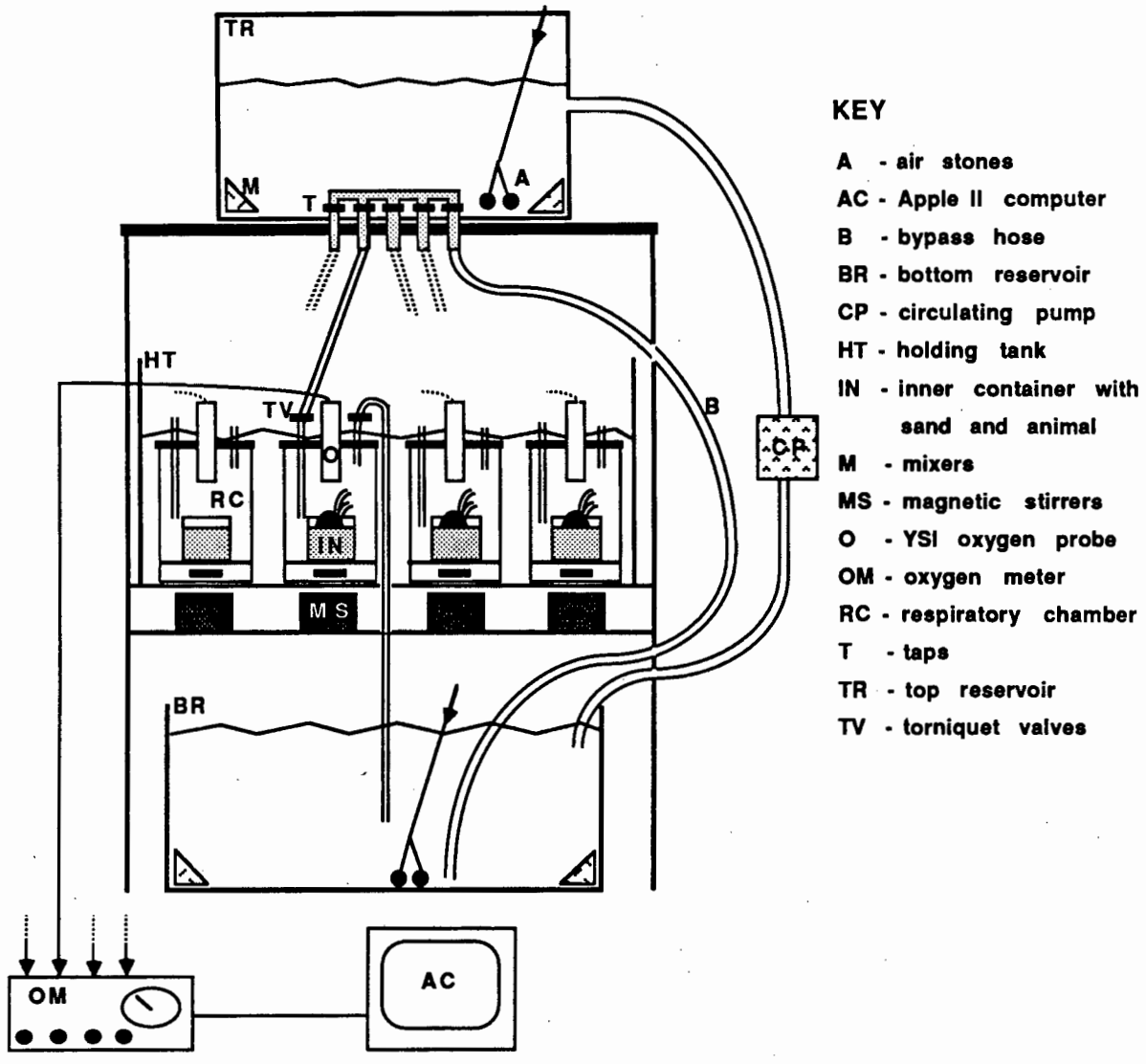

Fig. 5.1. Flow-through respirometer used to measure oxygen consumption in *D. serra*

individuals <15 mm shell width (approximately 0.16 g DW), two or three animals were placed in a respiration chamber with the resultant O_2 consumption being divided by the number of individuals displaying normal behaviour. The volume of sand was such that the siphons of buried *D. serra* were always freely exposed to water circulating in the chamber. Potential oxygen uptake by microbes in the sand and by the probes themselves was monitored by a control chamber with an inner container filled with sand but no animal. These chambers were flushed for 20 minutes with air-saturated sea water before being shut off to monitor oxygen decline.

The YSI probes were calibrated against the oxygen content of air-saturated sea water as determined by Winkler titration (Strickland & Parsons, 1968) at 15°C and 34°/oo. A data logger recorded readings simultaneously from each of the four probes every 30 seconds for 30 mins or until oxygen levels declined to 80% saturation. Oxygen consumption was graphically and digitally displayed on the monitor to enable rapid detection of erroneous runs. Data were stored and subjected to least-squares regression analysis of time (hr) against O_2 concentration (ml l^{-1}) with the slope equal to oxygen uptake (ml hr^{-1}) once control values were subtracted. This procedure allowed for a measure of respiration rate every 40 to 50 mins for each of the three experimental animals per run. After experiments, dry tissue weight (W; g) was determined by drying the animals at 60°C for 3 days

so that allometric equations of the form RR (respiration rate; ml hr^{-1}) = $a \cdot W^b$ could be produced.

In experiments to measure metabolic rates in starved individuals and in those recently fed, the reservoirs were filled with 40 l of 0.45-um filtered sea water which was used for a maximum of 8 runs and then replaced. Rate measurements during the "feeding" condition were determined by adding algae or seafoam detritus (preparation described in Chapter 4) to the filtered sea water in the bottom reservoir at regular intervals. Concentrations were monitored by counting suspensions on a Coulter counter every 30 mins. While the desired algal concentration was relatively easy to maintain, that of detritus was difficult due to settlement in the reservoirs and hoses of the flow-through system. Thus it was only possible to hold detrital concentrations over a range of particle densities, namely 20 to 30 X 10^6 l^{-1}. During experiments a control chamber served to monitor any O_2 change attributable to algal and particle metabolism.

After adding food to the flow-through system, an experimental animal (always in the well-fed condition) was allowed to feed for 30 mins before isolating a chamber and monitoring O_2 consumption. Thereafter, the same animal was only used in one or two more runs. This procedure was followed to minimise the effect on oxygen consumption of pronounced variations in feeding activity, which are

associated with extended exposure to food, especially algae (see Chapter 4).

AMMONIA EXCRETION EXPERIMENTS

Rates of net excretion were estimated by measuring ammonia-N, since 60 - 90% of the nitrogen excreted by *D. serra* (Prosch & McLachlan, 1984; Cockcroft, 1986), as well as other marine bivalves (Lum & Hammen, 1964; Hammen, 1968; Allen & Garrett, 1971; Ansell & Sivadas, 1973), is in this form. Either starved or well-fed individuals were placed in acid-washed glass beakers containing 1 l of 0.45-um filtered sea water at 15°C. Sufficient sand for burial was also supplied after it had been thoroughly rinsed in filtered, low-nutrient sea water and heated to 60°C for 24 hrs to drive off residual ammonia. Excretion rates of feeding individuals were measured after introducing *T. suecica* and detritus into the beakers. Desired concentrations were maintained by counting 5-ml samples every 20-30 mins on the Coulter counter. Water was kept in circulation by means of air-lift pumps positioned just above the sand. For each run a beaker with sand but without an animal served as a control. In experiments with feeding animals, two controls were maintained, one as above and another with food, sand and no animal. Any change in ammonia concentration caused by algae or detritus could be accounted for by difference.

Duplicate 5-ml samples were taken from each beaker at 1-hr intervals for 5 hrs and passed through a GFC filter.

Ammonia in the filtrate was determined by the indophenol-blue spectrophotometric technique of Koroleff (1976) as modified by Mostert (1983). In this method, which employs phenol-hypochlorite and is sensitive to 0.0036-0.0071 uM NH_4-N, amino acids do not interfere in colour development (Hawkins *et al.*, 1983). Excretion rates were calculated over 5 hrs from regression slopes of ug NH_4-N $animal^{-1}$ against time (hr) for different body sizes. This enabled the determination of allometric equations of the form U (ug NH_4-N h^{r-1}) = $a \cdot W^b$ (W = dry tissue weight; g). It is acknowledged that the validity of estimating ammonia excretion rates is based on the assumption that any increase in N content represents excretion of assimilated N by *D. serra*. As pointed out by Bayne & Scullard (1977), this involves two potential errors; the incorporation of unassimilated N in the form of dissolved products in the faeces and/or mucus and also bacterial uptake of nitrogen. Such errors were minimised by filtering sea water and removing faeces immediately on emergence from the exhalent siphon.

O:N ratios, as atomic equivalents of oxygen consumed to nitrogen excreted (Corner & Cowey, 1968; Mayzaud, 1973; Bayne, 1976), were calculated from allometric equations for rates of respiration and ammonia excretion in animals that were starved, fed or feeding on either algae or detritus. Such a ratio is indicative of the catabolic balance between protein, carbohydrate and lipids (Conover & Corner, 1968).

Ideally, O:N ratios should be assessed by simultaneously measuring O_2 uptake and ammonia excretion (Bayne, 1976; Bayne & Scullard, 1977a), but the design of the flow-through respirometry did not allow such a procedure.

RESULTS

OXYGEN CONSUMPTION

Table 5.1 lists the *a*- and *b*- values of the allometric equations relating oxygen consumption to body size in individuals which were starved, well-fed and feeding on different rations of algae and seafoam detritus. The resultant regressions are illustrated in double logarithmic plots to the base 10 in Fig. 5.2. Equality between slopes was assessed by covariance analysis and the Newman-Keuls (NK) multiple range test was used to verify differences between *a*-values (Table 5.2). Application of Student-*t* analysis identified significant differences in respiration rates when feeding on algae on the one hand and detritus on the other (Table 5.2).

With respect to each diet, there was no significant difference between regression slopes for starved, fed and feeding individuals, a common *b*-value (b_c) of 0.719 being computed for algae and 0.671 for detritus. Respiration rate thus clearly increased with animal size and the highly significant ($P < 0.005$) Pearson product-moment correlation

coefficients (r) demonstrate the strength of this relationship (Table 5.1). There was, however, a significant difference between *b*-values for RR while algae-feeding and *RR* while detritus-feeding (Student-*t* test, Table 5.2). This indicates that dietary quality influences the relationship between body weight and oxygen uptake. The detritus-regression shows a gentler slope. This difference in slopes precluded a significant testing of differences in elevations (*a*-values). However, in terms of weight-specific O_2 consumption, it was obvious that during filtration of detritus, less oxygen was used (0.3 ml hr^{-1} g^{-1}) than when filtering algae (0.4 - 0.5 ml hr^{-1} g^{-1}) of an equivalent quantity (Table 5.1).

The *a*-values (Table 5.1 and Fig. 5.2) indicate that O_2 consumption was significantly depressed (NK test, Table 5.2) by approximately 27% in starved individuals compared to well-fed ones. Filter-feeding when on an algal diet stimulated a 34%-41% increase in O_2 uptake relative to starved individuals and a 10%-20% increase over the rates of fed animals. Increasing the algal ration from 10 X 10^6 cells l^{-1} to 20 and 30 X 10^6 l^{-1} promoted a marginal, but significant increase in O_2 consumption. There was however, no significant difference between respiration rates at 20 and 30 X 10^6 cells l^{-1}.

When detritus was supplied during experiments to measure RR while feeding, rates were significantly lower than in either starved of fed individuals. This seems

Table 5.1. The *a* and *b* values from the allometric equation $RR = a \cdot W^b$ describing the relationship between aerobic respiration rate (RR in ml hr^{-1}) and body size (W in g DW) for different metabolic conditions: starved, fed and while feeding on *T. suecica* concentrations of 10, 20 and 30 X 10^6 l^{-1} and detrital foam between 20 and 30 X 10^6 particles l^{-1}.

Metabolic condition	*a*	*b*	n	*r*
Starved	0.27	0.69	44	0.99
Fed	0.37	0.70	44	0.98
Algae-feeding				
10 X 10^6	0.41	0.73	44	0.99
20 X 10^6	0.45	0.76	44	0.98
30 X 10^6	0.46	0.72	44	0.99
Detritus-feeding				
20-30 X 10^6	0.30	0.63	44	0.98

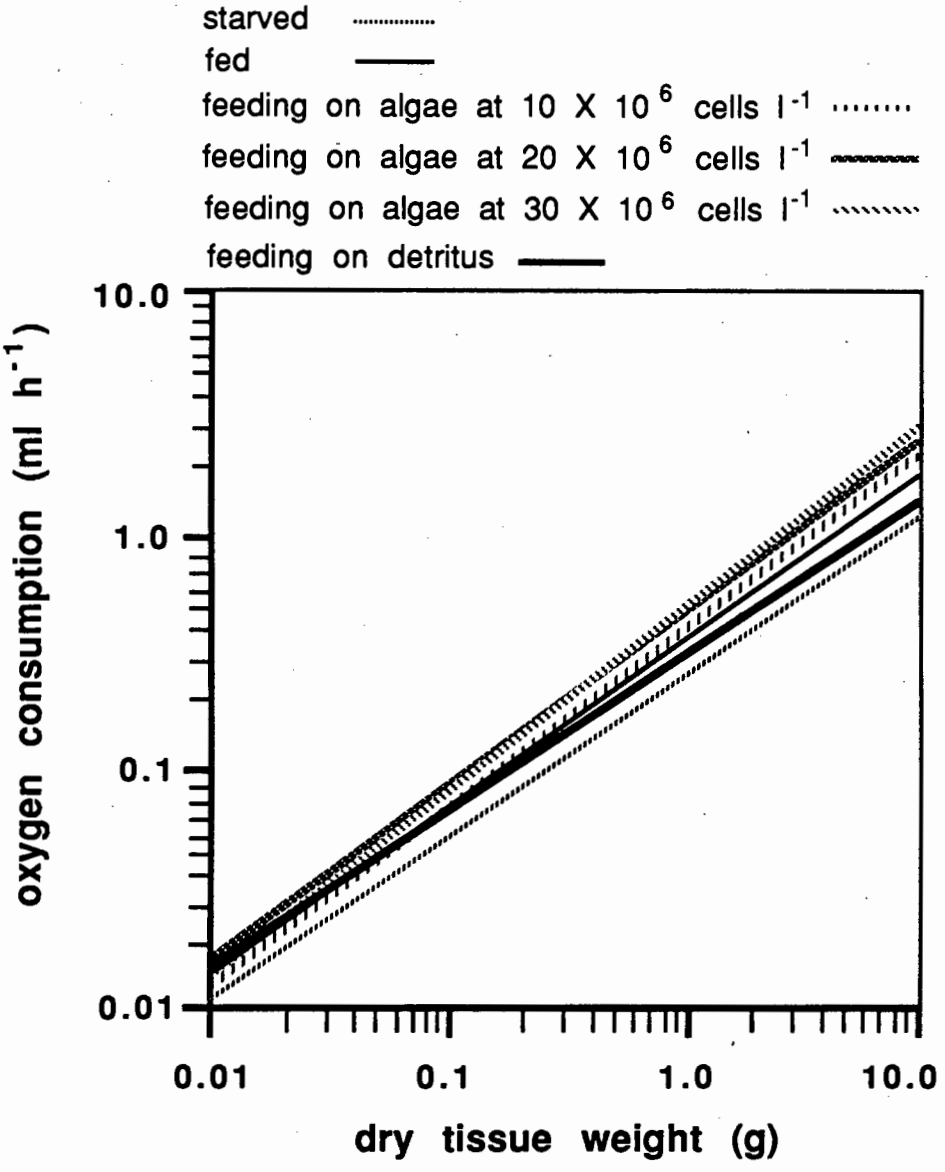

Fig. 5.2. Relationship between body size (g dry tissue weight) and oxygen consumption (ml h^{-1}) in *D. serra* which had been starved, fed and which were feeding on *T. suecica* at concentrations of 10, 20 & 30 X 10^6 cells l^{-1} and detritus in the range 20 - 30 X 10^6 particles l^{-1}. Allometric equations for lines are presented in Table 5.1.

Table 5.2. Analysis of covariance and multiple range testing between respiration rates (ml hr^{-1}) for different nutritional conditions in *D. serra*: starved (S), fed (F) and feeding on algae at three concentrations, 10 (A1), 20 (A2) and 30 (A3) X 10^6 *T. suecica* cells l^{-1} and detrital foam between 20 and 30 X 10^6 particles l^{-1} (D1). Analysis procedure follows Zar (1982) using $\log_{10}$ transformed data.

ANALYSIS OF COVARIANCE $P < 0.01$

	T. suecica Between S, F, A1, A2 & A3					Detrital foam Between S, F & D1				
	k	DF	F_s	F	b_c	k	DF	F_s	F	b_c
b-values	5	210	2.08	3.41	0.719	3	126	2.99	4.78	0.671
a-values	5	214	94.20	3.41		3	128	57.31	4.78	

NEWMAN-KEULS MULTIPLE RANGE TEST
(significant difference between a-values)

Algae Pairs	q	p	$q_{0.01,210}$	Detritus Pairs	q	p	$q_{0.01,210}$
S & F	14.11	2	3.64	S & F	14.41	3	4.20
S & A1	18.56	3	4.12	S & D1	3.17	2	3.70
S & A2	23.77	5	4.60	F & D1	11.20	2	3.70
S & A3	23.76	4	4.40				
F & A1	4.45	2	3.64				
F & A2	9.66	4	4.40				
F & A3	9.65	3	4.12				
A1 & A2	5.21	3	4.12				
A1 & A3	5.20	2	3.64				
A2 & A3	0.01	2	3.64				
Overall conclusion: A2 = A3					Overall conclusion: S = D1		

STUDENT-*t* TEST
Comparing regressions when feeding on algae and detritus

Food concentrations		Between slopes (*b*)			
T. suecica	detritus	t_s	$t_{0.01(2)}$	DF	
A1	D1	4.17	$\pm$2.66	84	Slopes all
A2	D1	4.32	$\pm$2.66	84	significantly
A3	D1	3.41	$\pm$2.66	84	different

ambiguous in view of the distinct increase in O_2 uptake associated with the presence of similar quantities of algae (10 - 30 X 10^6 particles l^{-1}). This difference cannot be attributed to food quality *per se*, but rather to the partial retraction and closure of the inhalent and exhalent siphons in response to the presence of detritus, a behaviour known markedly to reduce clearance rates (see Chapter 4). Starved individuals displayed similar, but less pronounced quiescence, whilst well-fed and algae-feeding individuals continuously maintained open and extended siphons in the respiration chambers. It appears that in some way postural changes in the siphons concomitant with changes in clearance rates (and ventilation rates if the two are assumed to be synonymous - see Bayne *et al.*, 1976; Jorgensen *et al.*, 1986) influence changes in oxygen consumption.

Respiration rates can also be viewed in terms of the energetic expenditure of standard metabolism relative to the physiological cost of digestion and assimilation and the mechanical cost of filtration (see Warren & Davies, 1967; Bayne *et al.*, 1976; Bayne & Scullard, 1977b). Physiological costs were assumed to be represented by rates of well-fed individuals minus standard metabolism and the mechanical cost of filtration, by rates while feeding minus standard metabolism plus digestion and assimilation.

The proportional utilisation of energy available in ingested and absorbed rations in these different processes has been calculated in Table 5.3 for 1 g of dry tissue

weight. Data on food intake and absorption at 10, 20 & 30 X 10^6 algal cells l^{-1} and 20-30 X 10^6 detrital particles l^{-1} were derived from Tables 4.6 and 4.9 respectively (Chapter 4). Irrespective of dietary quantity or quality, most of the energy available in the ingested and absorbed ration was used in standard metabolism, followed by the cost of digesting and assimilating food (often referred to as the specific dynamic action [SDA] of the ration - see Bayne & Scullard, 1977b). Very little of the energy in food was used for filtering particulates from suspension.

At 10 X 10^6 algal cells l^{-1}, 33% of the energy in the ingested ration and 60% of that in the absorbed ration was used in standard metabolism (Table 5.3). With an increase in concentration to 20 - 30 X 10^6 cells l^{-1}, the proportional cost of standard metabolism declined to between 5 - 10% of the ingested and absorbed ration. At a detrital concentration of 20 - 30 X 10^6 particles l^{-1}, the proportional utilisation of energy for maintenance, digestion and assimilation was similar to that shown at the lowest algal ration. Because RR of detritus-feeding animals was less than for starved or fed animals, negative values resulted in the calculation of mechanical costs of feeding. This however, should not be seen in absolute terms but rather as an indication that the digestive and assimilation of detrital material was energetically more expensive than clearance of the particles from suspension.

Table 5.3. The energetic costs of standard metabolism and the processes of feeding (mechanical cost), and digesting and assimilating food (physiological cost) expressed as percentages of the energy available in ingested (IR) and absorbed (AR) rations of algae and detritus. Data for IR and AR as J hr^{-1} g^{-1} were derived from Tables 4.6 and 4.9 respectively.

Conc	Ration		Percentage of IR			Percentage of AR		
$X10^6$ l^{-1}	J hr^{-1} g^{-1} IR	AR	Stand Metab	Mech Cost	Phys Cost	Stand Metab	Mech Cost	Phys Cost
Algae								
10	16.97	9.32	32.81	4.43	11.85	59.75	8.07	21.58
20	94.19	56.51	5.91	1.66	2.14	9.85	2.77	3.56
30	107.64	76.18	5.17	1.64	1.87	7.31	2.32	2.64
Detritus								
20-30	16.05	7.23	34.70	---	12.53	77.03	---	27.81

Energy expended in standard metabolism and mechanical and physiological processes can be further analysed in terms of percentage costs of routine metabolism (=100%) for each diet and for a representative size range of *D. serra* (0.1 - 5.0 g) [Table 5.4].

When algae was used as food, the proportion of overall metabolism representing basic maintenance decreased from 71 - 54% with an increase in the size of *D. serra*. On a detrital diet, a reverse trend with respect to size was evident. Virtually 100% of total metabolism in large individuals was ascribed to standard processes whereas the corresponding percentage in smaller animals was 80%. The same diet-specific size-related trends were observed in the metabolic fraction relating to physiological costs. With an increase in size, there was a decline from 24% to 21% on an algal diet and an increase from 27% to 39% with detritus as food.

A greater portion of routine metabolism in large individuals represented the cost of filtering algal cells (26%) than in small ones (5%) [Table 5.4]. It was not possible to calculate similar proportional costs for clearing detrital particles for reasons already given with reference to Table 5.3.

A comparison of respiration rates while feeding on algae and detritus with corresponding clearance rates (l hr^{-1}) (Chapter 4) allows for the calculation of O_2 extraction efficiencies and convection requirements (see Shumway,

Table 5.4. Energy used in respiration expressed proportionally in terms of the cost of standard and routine metabolism for different sized animals as calculated from the regression equations in Table 5.1. AD = algal diet, DD = detrital diet. In the case of algae, the regression equation for respiration rates while feeding at 20 X 10 ^{6} cells l $^{-1}$ was applied here. A mean oxycalorific equivalent of 20.08 J per ml oxygen consumed was assumed (Gnaiger, 1983).

BODY SIZE	OXYGEN CONSUMPTION				ENERGY USED IN ROUTINE METABOLISM (= 100%)										
dry tissue weight (g)	standard metabolism (ml h^{-1})	standard metabolism + digestion + assimilation (ml h^{-1})	standard metabolism + digestion + assimilation + feeding = routine metabolism (ml h^{-1}) AD	DD	Standard metabolism J h^{-1}	% AD	% DD	Feeding J h^{-1} AD	DD	% AD	DD	Digestion + Assimilation J h^{-1}	% AD	% DD	Routine metabolism J h^{-1} AD	DD
0.100	0.056	0.075	0.079	0.070	1.138	70.86	79.97	0.081	—	5.04	—	0.386	24.03	27.13	1.606	1.423
0.500	0.170	0.230	0.268	0.192	3.455	63.43	88.54	0.772	—	14.17	—	1.219	22.38	31.24	5.447	3.902
1.000	0.274	0.373	0.454	0.297	5.569	60.36	92.26	1.646	—	17.84	—	2.012	21.81	33.33	9.227	6.036
2.000	0.441	0.605	0.768	0.459	8.962	57.42	96.08	3.313	—	21.23	—	3.333	21.35	35.73	15.608	9.328
4.000	0.709	0.980	1.300	0.710	14.409	54.42	99.86	6.503	—	24.61	—	5.508	20.85	38.17	26.420	14.429
5.000	0.827	1.145	1.540	0.817	16.807	53.70	101.22	8.028	—	25.65	—	6.463	20.65	38.92	31.300	16.604

1983). It is necessary to assume in such calculations that clearance rates approximate those of ventilation, an assumption which is valid if filtration efficiency is high (Bayne *et al.*, 1976), as has been shown in *D. serra* (Matthews *et al.*, 1989).

Table 5.5 shows the allometric equations relating O_2 uptake, clearance rate, convection requirements (CREQ; amount of water pumped/amount of oxygen consumed) and extraction coefficients (E; amount of oxygen used/amount of oxygen available) to concentrations of algae and detritus. The weight coefficients show that metabolism increased faster in relation to body size than did pumping rate when on an algal diet. With detritus however, the size-dependent increment of these two rates were in close agreement. This contrast is naturally reflected in the relationship between body size and convection requirements/O_2 utilisation efficiency. When clearing algae from suspension, CREQ declined and E increased with increase in size but with detritus there was only a weak indication of such trends (CREQ slope = -0.01 and E slope = +0.01).

The Y-intercept values (*a* and *c* in Table 5.5) indicate the marked decline in pumping rate with a fall in algal concentration from between 20 - 30 X 10^6 cells l^{-1} to 10 X 10^6 l^{-1}, a decrease which was accompanied by only a moderate, but significant (Table 5.2), drop in O_2 consumption. This can be seen as a reduction in ventilation rate of 62% coincident with a reduction in O_2 uptake of

Table 5.5. Equations relating oxygen consumption (RR; ml hr^{-1}), clearance rate (CR; l hr^{-1}), convection requirement (CREQ; l sea water pumped ml O_2 available^{-1}) and the extraction coefficient (E; ml O_2 consumed ml O_2 available^{-1}) to dry body weight (W; g) where RR = aW^b; CR = cW^d; CREQ = CR/RR ($[c/a]W^{[d-b]}$); E = RR/CRx($[a/cx]W^{[b-d]}$). x is the initial amount of oxygen available (ml dissolved O_2 l sea water^{-1}). Calculation procedure adapted from Shumway (1983).

Food Conc $X10^6$ l^{-1}	RR ml O_2 h^{-1}		CR l hr^{-1}		CREQ		E	
	a	*b*	*c*	*d*	*c/a*	*(d-b)*	*a/cx*	*(b-d)*
Algae								
10	0.41	0.73	0.40	0.49	0.98	-0.24	0.18	0.24
20	0.45	0.76	1.05	0.55	2.33	-0.21	0.07	0.21
30	0.46	0.72	0.99	0.49	2.15	-0.23	0.08	0.23
Detritus								
20-30	0.30	0.63	0.31	0.62	1.033	-0.01	0.17	0.01

between only 9% and 11%. This contrast arises because, with the reduction in ventilation rate, the extraction efficiency of oxygen increased from around 8% to 18%. Such high utilization efficiency was also observed under conditions of reduced pumping with detritus. Naturally, high E values were coincident with low convection requirements and vica versa.

AMMONIA EXCRETION

The relationship between ammonia excretion (*u*g NH_4-N hr^{-1}) and body size (g DW) in starved, fed and feeding animals is given as allometric equations in Table 5.6 and plotted on a double log scale in Fig. 5.3. For all nutritional conditions there was a positive correlation between size and excretion rates which proved highly significant by the Pearson Product-moment correlation coefficients ($P < 0.005$). Excretion rates were fairly constant during the 5-hr experiments, the little temporal variability that did occur being most evident in starved individuals ($r = 0.77$, Table 5.6).

The same statistical procedure as used on respiration data (Table 5.2) was applied in analysing the significance of the effect of body size, nutritional condition and dietary quality on U (Table 5.7). The *b*-values were significantly the same indicating a consistent relationship between animal size and ammonia release irrespective of

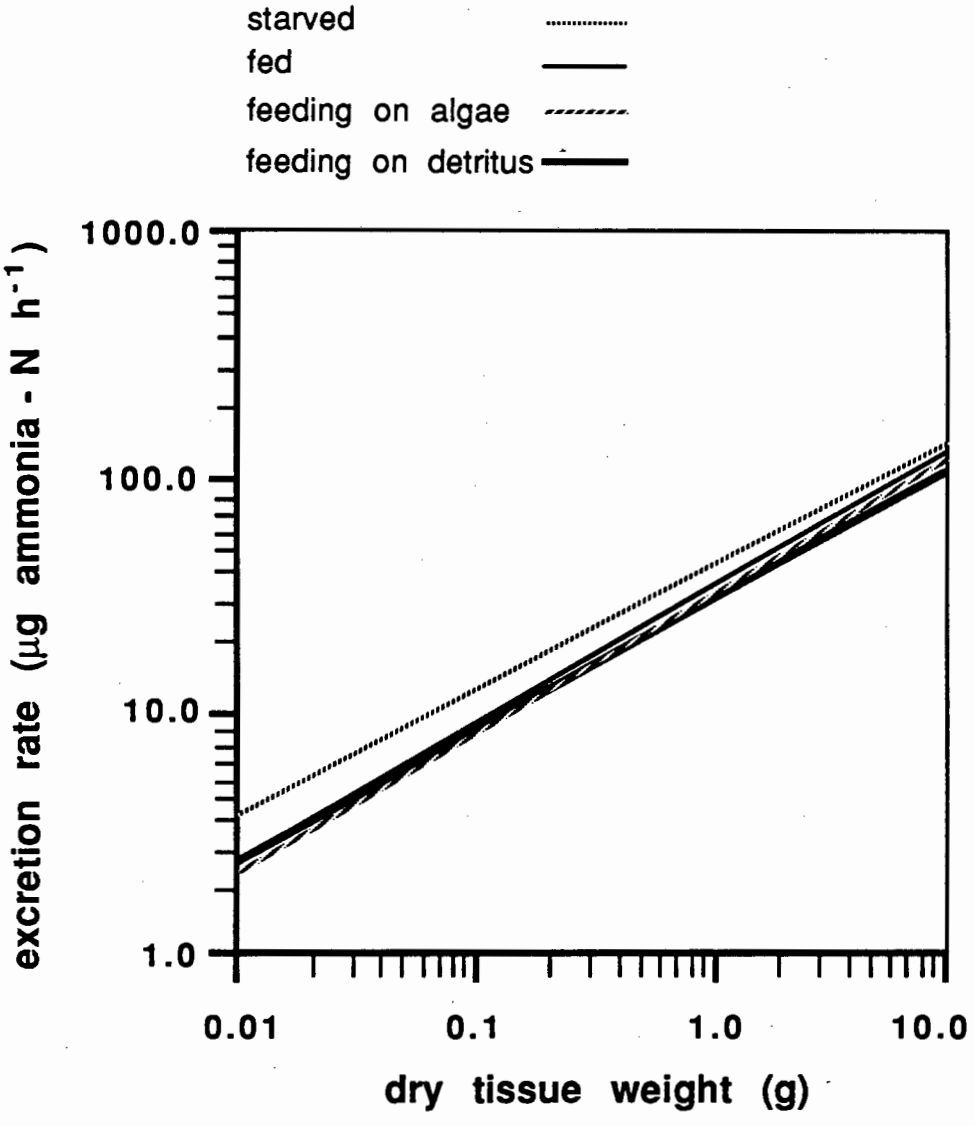

Fig. 5.3. Relationship between body size (g dry tissue weight) and excretion rate (µg ammonia - N h^{-1}) in *D. serra* which had been starved, fed and which were feeding on algae and detritus at concentrations of approximately 20 X 10^6 particles l^{-1}. Allometric equations for lines are presented in Table 5.6.

nutritional status or diet. A common slope of 0.537 (b_c) was thus computed.

Table 5.6. *a*- and *b*-values from the allometric equation $U = a \cdot W^b$ describing the relationship between ammonia excretion (U in ug NH_4-N hr^{-1}) and body size (W in g DW) of *D. serra* which were starved, well-nourished and feeding on algae and detritus at concentrations of approximately 20 X 10^6 particles l^{-1}.

Metabolic condition	*a*	*b*	n	r
Starved	41.27	0.49	25	0.77
Fed	35.42	0.55	25	0.83
Algae-feeding	33.42	0.56	25	0.84
Detritus-feeding	31.12	0.51	25	0.90

Table 5.7. Analysis of covariance and multiple range testing between excretion rates (ug NH_4-N hr^{-1}) when *D. serra* was starved (S), fed (F) or feeding on algae (A) or detritus (D) at concentrations of 20 X 10^6 particles l^{-1}. Analysis procedure follows Zar (1982) using $\log_{10}$ transformed data.

ANALYSIS OF COVARIANCE $P < 0.01$

Between S, F, A, D

	k	DF	F_s	F	b_c
b-values	4	92	0.17	3.98	0.537
a-values	4	95	7.04	3.98	

NEWMAN-KEULS MULTIPLE RANGE TEST
(significant difference between *a*-values)

Pairs	q	p	$q_{0.01,92}$
S & F	2.03	2	3.73
S & A	3.27	3	4.24
S & D	6.38	4	4.55
F & A	1.19	2	3.73
F & D	4.29	3	4.24
A & D	3.08	2	3.73

Overall conclusion:
S = F = A, A = D

Excretion rates were greatest during starvation (41 ug NH_4-N hr^{-1} g^{-1}), a little less when fed (35 ug hr^{-1} g^{-1}) and least when feeding on algae (33 ug hr^{-1} g^{-1}) and detritus (31 ug hr^{-1} g^{-1}). However, there was no significant difference in rates when starved, fed and feeding on algae or between rates while algae- and detritus-feeding (NK test). Ammonia release while filtering detritus on the other hand was significantly less than rates in starved and fed animals. This contrasts with the similarity between "starved/fed"-rates and those while algae-feeding. This serves to highlight once again the effect of dietary quality on pumping rates. As reduction in movement of water into and out of the mantle cavity is coupled to reduced metabolic expenditure, this would undoubtedly depress the rate of ammonia excretion.

Changes in the rate of ammonia excretion are best understood when related to aerobic metabolism by means of the O:N ratio. Table 5.8 and Fig. 5.4. show that such ratios are positively correlated with body size, a relation which is to be expected since weight coefficients for ammonia excretion with respect to nutritional condition and diet were less than corresponding respiratory coefficients (compare Tables 5.1 & 5.6). However, resultant *b*-values were low (0.20 and less) so that there was only an approximate doubling of ratios with a 50-fold increase in size.

Table 5.8. O:N ratios in relation to body size (g DW) when *D. serra* was starved, fed or feeding on algae or detritus. The ratios are derived from the allometric equations for rates of respiration (Table 5.1) and excretion (Table 5.5).

Body Size	Condition of animal			
(g DW)	Starved	Fed	Algae Feeding	Detritus Feeding
0.100	5.155	9.238	10.603	9.134
0.500	7.114	11.760	14.644	11.081
1.000	8.227	13.049	16.821	12.041
2.000	9.388	14.479	19.323	13.088
4.000	10.784	16.065	22.195	14.221
5.000	11.270	16.612	23.207	14.608
Allometric Equations	$8.18 \cdot W^{0.20}$	$13.05 \cdot W^{0.15}$	$16.82 \cdot W^{0.20}$	$12 \cdot 04W^{0.12}$

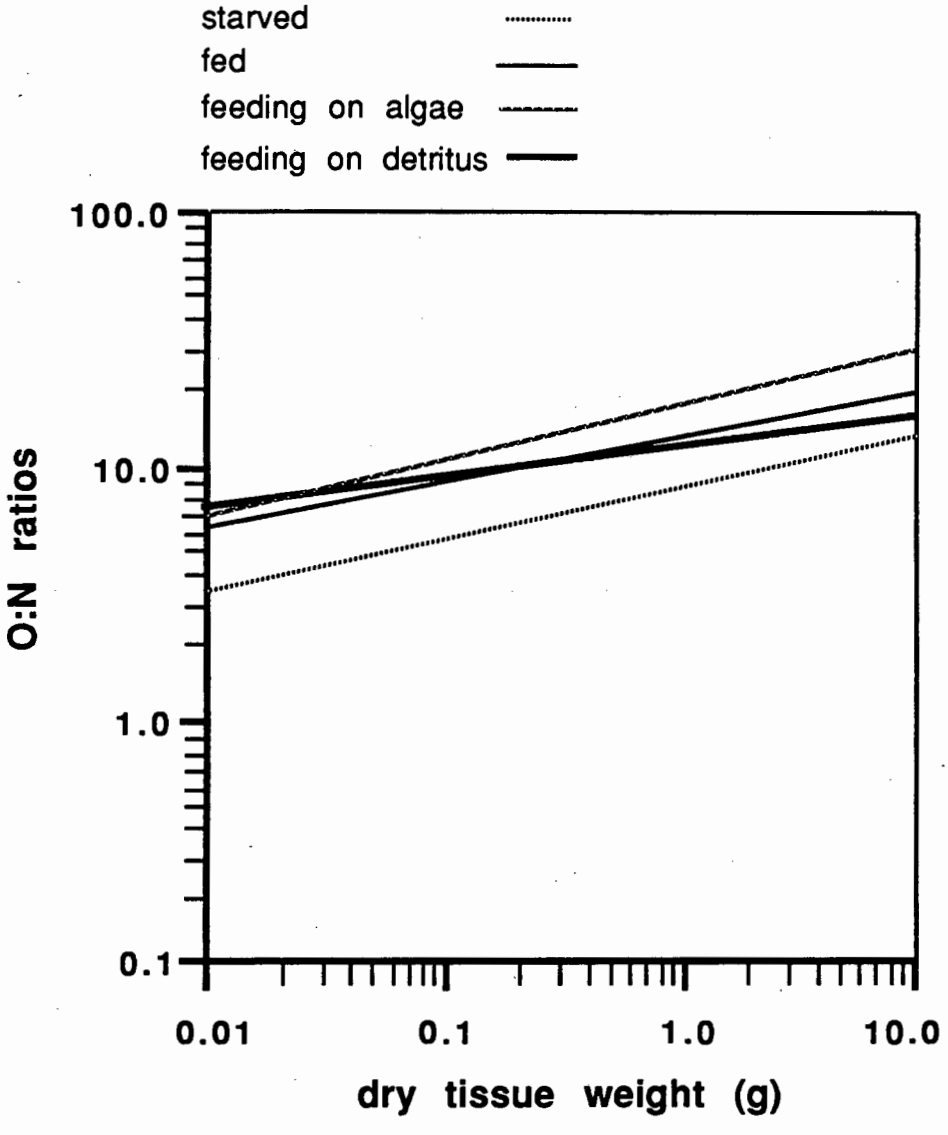

Fig. 5.4. Relationship between body size (g dry tissue weight) and O:N ratios in *D. serra* which had been starved, fed and which were feeding on algae and detritus at concentrations of approximately 20 X 10^6 particles l^{-1}. Allometric equations for lines are presented in Table 5.8.

The lowest O:N ratios (<11.3) were recorded in starved individuals. These animals showed the maximum ammonia excretion rate together with the minimum rate of O_2 uptake. Such ratios would be indicative of a greater proportion of protein catabolism relative to carbohydrate and lipid (Conover & Corner, 1968, Bayne & Newell, 1983). Ratios increased slightly in fed animals, the magnitude of the increase being greater in the smaller individuals. Excretion relative to metabolic activity while feeding on detritus resulted in ratios similar to that of fed *D. serra*, signifying a shift toward carbohydrate utilisation in these animals. During algae-feeding, lower excretion rates but greater oxygen consumption resulted in maximum ratios (approximately 23.2) indicating a further move towards carbohydrate and lipid metabolism.

DISCUSSION

The *b*-values relating size of *D. serra* (g DW) to oxygen consumption rate (ml hr^{-1}) when starved, fed and feeding (0.63-0.76) were well within the range of 0.44-1.09 reported by Bayne & Newell (1983) for 10 species of particulate-feeding bivalves. In addition, an overall mean slope of 0.71 matched that of 0.70 calculated by these authors, as well as by Bayne (1976), from published data for animals displaying a wide range of activities. The mean also approximated the theoretical value of 0.75 for poikilotherms suggested by Hemmingsen (1960). A single value of *b* cannot, however, be accepted as a species characteristic as indicated by the significant difference between slopes of body size versus respiration rates while feeding. The physiological implications of differences in intraspecific weight exponents must be recognised.

Statistical analysis further revealed that any change in O_2 uptake resulting from the nutritional condition of an individual or from changes in food quantity, was proportionally the same for all sizes of animals. However, with a contrast in dietary quality, weight-related O_2 consumption changed significantly so that larger animals relative to smaller ones respired more slowly when feeding on detritus (9.91 J·mg DW^{-1}) compared to a more enriched algal diet (20.70 J·mg DW^{-1}). Dietary quality has not previously been reported to affect allometry among bivalves. More commonly, intraspecific variation in slope is

attributed to changes in temperature, salinity, ration level and activity patterns not associated with the nature of the diet (Newell & Roy, 1973; Thompson & Bayne, 1974; Widdows, 1978a; Griffiths & King, 1979a; Shumway, 1983), as well as with seasonal reproductive cycles (Worrall *et al.*, 1983).

Significant differences in oxygen consumption between starved, fed and feeding individuals made it possible to resolve the metabolic components associated with basic maintenance plus the mechanical process of filter-feeding and the physiological cost of digesting and assimilating food. Irrespective of the quality or concentration of food or whether data were expressed as a proportional cost of routine metabolism or of the energy value of the ingested/absorbed ration, standard metabolism rather than feeding activity utilised the most energy. With respect to feeding, post-ingestive processes were more costly than filter-feeding.

The above partitioning of metabolic activity contrasts with that reported for *Mytilus californianus* (Bayne *et al.*, 1976) and *Mytilus edulis* (Bayne & Scullard, 1977b) in which mechanical costs were by far the greatest, followed by maintenance and then physiological expenses. In *M. californianus* (1-g DW), filter-feeding accounted for 59% of routine metabolism. This was equivalent to 18% of the value of the food ingested with only 6% being used for physiological processes. Very similar results were recorded for *M. edulis* (24% and 4% respectively). On the other hand,

in a study on *Mytilus chilensis* (Navarro & Winter, 1982), partitioning of the routine rate of oxygen consumption was similar to that assessed here for *D. serra*. With an increase in the weight of *M. chilensis* from 0.2 to 3.0 g DW, maintenance, mechanical and physiological costs ranged from 68-75%, 3-8% and 29-17% of routine rate. It is thus apparent that mechanical costs were exceedingly low compared to the other studies in which filtration caused the standard rate to at least double. Recently, Clemmesen & Jorgensen (1987) measured the O_2 consumption of the gills in *M.edulis* and found this to account for no more than 15% of total metabolism.

The probable reason for the above differences lies in contrasting methodology. In the work of Bayne & Scullard (1977b), the proportionate cost of filter-feeding was assessed from the rate of oxygen uptake immediately on the presentation of food to starved individuals. This approach resulted in a surge in pumping activity which promoted an increase in respiration. Bayne *et al.* (1976) regressed filtration rate against O_2 uptake in starved and fed mussels to measure the cost of particle clearance. In this study, and that of Navarro & Winter (1982), feeding costs were determined from oxygen consumption monitored in fed animals presented with a steady food supply for an extended period.

The relative partitioning of available energy between maintenance and feeding thus depends on the nutritional state of the bivalve. When a quiescent, starved animal is

provided with food, increased oxygen consumption rates represent the demands of abrupt commencement of pumping plus associated postural movements, as well as increased activity of the digestive system. In contrast, increased O_2 uptake in fed animals correlates with only a moderate rise in filtration and post-ingestive activity. Interpretation can be further complicated by the storage cycle in body tissues. Widdows (1978a) found that as long as sufficient reserves were available to meet the energy demands of gametogenesis in *M. edulis*, reduction in metabolic rate during starvation is prevented.

The point at which, and the duration over which, oxygen uptake is monitored once food is presented to an animal is also a crucial factor in measuring feeding costs. Fed *D. serra* were allowed to feed for 30 mins before O_2 uptake was measured for a maximum of two 30-min periods per individual. Immediate monitoring on addition of food can lead to a 2-3 fold increase in oxygen consumption compared to standard rates of metabolism (Thompson & Bayne, 1972; Newell, 1976, 1979; Bayne & Scullard, 1977b; Griffiths & King, 1979a). Furthermore, since fluctuating filtration rates are characteristic of bivalves exposed to a steady food supply for an extended period (Bayne & Newell, 1983; Hawkins *et al.*, 1985; Chapter 4), respiration rates may reflect concomitant variation if the two are assumed to be correlated (Bayne *et al.*, 1976).

An interesting feature in this study was the effect of dietary quality, not only on the allometric relations of respiration rates (*b*-values) but also on the absolute rate of oxygen consumption (*a*-values). Feeding on algae initiated an increase in O_2 uptake 34-41% greater than in quiescent animals, whereas on a detrital diet there was only a 10% increase over standard rates.

Contrasting diets have been used in previous research into respiration rates, but no significant effects of food quality on the rates of O_2 uptake have been reported. Thompson & Bayne (1972) found that on presentation of food to *M. edulis*, oxygen consumption increased to the same extent whether the food was particulate or non-particulate (soluble extracts). Shumway (1983) demonstrated the same type of response in *Mulina lateralis* using algae and charcoal. In a seasonal assessment of physiological rates in *M. edulis*, Bayne *et al.* (1987) found O_2 uptake was sometimes depressed with a reduction in the organic content of food, but this trend was inconsistent. Other studies have shown that the silt concentration of suspended particulates has no effect on respiration rate (Widdows *et al.*, 1979; Mohlenberg & Kiorboe, 1981; Kiorboe *et al.*, 1981). In the kelp-bed mussel, *A. ater*, rates remained unchanged whether food was cultured algae (Griffiths & King, 1979a) or kelp detritus of varying quality (Stuart, 1982). A depression in uptake consistent with a change in diet from field to laboratory foods has been reported (Bayne & Newell,

1983), but this, like the examples above, has not been conclusively linked to dietary quality.

Reduction in the respiration rate of *D. serra* in the presence of detritus was clearly coincidental with a decrease in filtration rate. This rate is, in turn, equivalent to ventilation rate, since the efficiency with which *D. serra* retains suspended particulates is high (Matthews *et al.*, 1989). In these experiments this was apparent from reduced valve gape and partial retraction of the siphons, postural changes which indicate reduced ventilation (see Chapter 4). Recently Jorgensen *et al.* (1988) have quantified the fall in pumping pressure and flow rate with a reduction in valve gape in *M. edulis*. Retraction of mantle edges and siphons at reduced valve gape resulted in a reduction in the width of interfilament canals and thus in the distance between opposing bands of lateral cilia. The authors conclude that this distance is the main factor determining rate of water transport, rather than any physiological regulated process.

In an earlier publication, Jorgensen *et al.* (1986) have warned against seeking a causal relation between rates of oxygen consumption and ventilation, concluding that any correlation reflects physical conditions of viscous flow and diffusion boundary layers rather than mechanical costs of water transport. At low ventilation rates the boundary layer is thick but diffusional pathways within the tissues tend to be short thereby enhancing the efficiency with which

oxygen is extracted (E) from the ventilation current. For *D. serra* such an efficiency amounted to 17% when filtering detritus, compared to only 8% in the presence of 20-30 X 10^6 algal cells l^{-1}. The former extraction efficiency is high relative to values in the literature for filter-feeding bivalves which usually fall in the range of 1 - 10% (Jorgensen, 1975; Magnum & Burnett, 1975; Ansell & Sivadas, 1973; Shumway, 1983). Jorgensen *et al.* (1986) regard a high extraction efficiency as indicative of measurements made on sub-optimally ventilating animals. This is probably applicable to *D. serra* during detritus-feeding as well as when pumping at low algal rations when E was also very high (18%). On the other hand, a lower extraction efficiency, as when feeding at moderate algal rations, is indicative of water transport which exceeds oxygen requirements but which enables optimal clearance of the suspended food.

In *D. serra*, relationships between ammonia excretion rates and body size were remarkably consistent (b_c = 0.537) being unaffected by nutritional condition, feeding activity or quality of the diet. Other studies have not specifically addressed the influence of these factors but have rather focused on seasonal effects such as changes in temperature and food availability. In this regard some workers have found no significant differences in *b*-values with season (Widdows, 1978a; Jordan & Valiela, 1982; Hawkins *et al.*, 1985). On the other hand, Bayne & Scullard (1977a) have found exponents for *M. edulis* to vary seasonally between

0.482 and 1.480, such fluctuations probably stemming from differing metabolic priorities in reproductive and juvenile mussels. Thus,in the long-term, *b*-values for *D. serra* are likely to deviate from 0.537 and may, in addition, show less consistency with respect to nutritional status and diet during the course of gametogenic change.

As part of a study into the nitrogen budget of a sandy beach surf zone on the south coast of South Africa, on-site estimates of ammonia excretion by *D. serra* were very low, averaging 7.3 *ug* NH_4-N g^{-1} hr^{-1} (Prosch & McLachlan, 1984). Furthermore, attempts to monitor excretion in the laboratory provided negative results. In a separate study, Cockcroft (1986) measured rates ranging from 10 to 47 *ug* NH_4-N g^{-1} hr^{-1} for animals from the same population. This compares well with the rates of 31 to 41 *ug* NH_4-N g^{-1} hr^{-1} measured for *D. serra* from Ouskip beach.

Unfortunately, since the above publications did not provide sufficient information on experimental conditions, it is difficult to explain such a disparity in rates. Rates of ammonia release are affected by a variety of factors besides nutritional status. These include temperature, ration level, reproductive condition and phasic activity of the digestive gland (Ansell & Sivadas, 1973; Bayne & Scullard, 1977a; Widdows, 1978a; De Vooys & De Zwaan, 1978; Hawkins *et al.*, 1983; Chapter 7). Much of the variability in rates has been attributed primarily to the cycle of gametogenesis (Griffiths & Griffiths, 1987). Adult *D. serra*

used in excretion experiments were collected in December, a time of the year when there is the greatest frequency of spawning and post-spawned individuals (de Villiers, 1975a; Birkett & Cook, 1987; pers. obs.). According to studies in which excretion rates have been measured at different stages of the gametogenic cycle (Bayne, 1973; Widdows, 1978a; Worrall *et al.*, 1982; Hawkins *et al.*, 1983), such a condition in *D. serra* would coincide with moderate values for U, minimum U being associated with germinal quiescence and maximum U with gamete maturation. Such a cycle reflects seasonal shifts between a reliance on carbohydrate as a primary energy reserve during quiescence when glycogen storage is maximal, to a greater utilisation of protein during and immediately after spawning.

The O:N ratios of *D.serra* reflected considerable protein catabolism and were thus, with respect to adult individuals, consistent with the state of spawning. The ratios (8 to 17 for 1 g dry tissue weight) were closer to the theoretical maximum of 9.3 for pure protein catabolism (Bayne & Newell, 1983; Griffiths & Griffiths, 1987). Higher values of approximately 30 or more are indicative of greater proportional utilisation of carbohydrates and lipids (Ansell & Sivadas, 1973; Bayne & Scullard, 1977a; Worrall *et al.*, 1982; Hawkins *et al.*, 1983; Hawkins *et al.*, 1985).

Although the difference in ratios between starved, fed and feeding individuals was slight, a greater utilisation of protein as an energy substrate was suggested by the

recording of lowest ratios among starved animals. Increased excretion of ammonia during nutritional stress has also been measured in *M. edulis* (Bayne & Scullard, 1977a; Hawkins *et al.*, 1985), *Donax vittatus* (Ansell & Sivadas, 1973) and *Scrobicularia plana* (Worrall *et al.*, 1982). This, together with a tendency for oxygen consumption to decline during starvation, also resulted in low O:N ratios. However, it should be borne in mind that the effect of starvation is dependent on the nature of body reserves. Bayne & Scullard (1977a) found that when glycogen reserves were high in *M. edulis*, starvation actually reduced the rate of ammonia release and thereby elevated the O:N ratio.

Any discussion on metabolic expenditure by *D. serra* is not complete without acknowledging the important contribution of anaerobic respiration to total metabolism. The occurrence of anaerobiosis among marine bivalves was first detected from the accumulation of associated end products such as alanine, malate and succinate (De Zwaan *et al.*, 1975; De Zwaan, 1983). Of more importance to balancing energy budgets, however, has been the more recent development of simultaneous direct calorimetric and respirometry techniques in which the relative proportions of aerobic to anaerobic respiration can be measured (Hammen, 1979, 1980; Pamatmat, 1979; Famme *et al.*, 1981). These studies demonstrated that anaerobic respiration occurs even under fully oxygenated conditions, although its relative contribution to total metabolism can vary from 5% (Famme *et*

al., 1981; Shick *et al.*, 1983) to as much as 60% (Hammen, 1979, 1980).

While lack of facilities prevented the measurement of the ratio of aerobiosis to anaerobiosis in *D. serra*, evidence points to considerable anaerobic metabolism. Trueman & Brown (1987) recorded oxygen tensions in the pedal sinus of *D. serra* which never exceeded 50% of saturation and were sometimes less than 10% during periods of burrowing. These authors also noted that the pedal musculature lacked mitochondria except at the extreme outer surface of the foot, where some oxygen presumably diffuses inwards through the pedal wall. Supporting evidence of pedal muscles functioning anaerobically in this study was the absence of an increase in O_2 consumption during digging no matter how vigorous. With the use of biochemical techniques, extensive anaerobiosis has been quantified during the burrowing of other bivalves such as the razor clam *Ensis directus* (Schiedek & Zebe, 1987).

Another indication of anaerobic respiration in *D. serra* is the oxyconformity of the animal and the fact that it incurs an oxygen debt during hypoxia (Van Wijk *et al.*, 1989). Furthermore, *D. serra* reacts to unfavourable conditions by withdrawing totally into its shell (see Chapters 2 & 7); under these circumstances, no oxygen uptake could be measured and metabolism, although possibly greatly reduced, must be largely anaerobic. Such anaerobic

metabolism has been measured biochemically during valve closure in *M. edulis* (De Vooys, 1987).

Although the extent of anaerobiosis in *D. serra* is not known, it is likely to be of great adaptive significance in the turbulent surf-environment at Ouskip. Rapid burrowing and frequent repositioning in the sand would not initiate a sudden increase in the oxygen demand of muscular tissue. Anaerobiosis was probably not so important during this study in which animals were always submerged, buried and not exposed to excessive turbulence. Indirect evidence indicates that anaerobic respiration would be a major component of total metabolism during periods of tidal exposure and in maintaining a position in the surf. In the following chapter it becomes important to realise the potential for anaerobic metabolism in *D. serra* when the acquisition of energy is balanced against expenditure.

CONCLUSIONS

1.) Intraspecific variation in the relationship between respiration rate and body size in bivalves is most commonly attributed to the influence of environmental factors other than the nature of diet. This study is the first to identify the significant effect of food quality on this size-rate relationship. This focuses attention on the unacceptability of adopting a single weight coefficient as a species characteristic in the prediction, from allometric equations, of respiration rates while feeding.

2.) Standard metabolism, rather than feeding activity, accounted for most of the energetic expenditure associated with aerobic respiration in *D. serra*. This contrasts to the high feeding but relatively low maintenance costs recorded in other bivalve species. It is concluded that such differences are not necessarily species specific, but relate to nutritional condition and intrinsic fluctuations in ventilation (clearance) rates. It is also important to consider the point at which, and the duration over which, O_2 uptake is monitored once food is presented to an animal.

3.) Dietary quality had a significant effect on the respiration rate of individual *D. serra*. Rates were notably lower when feeding on a near-natural, but nutritionally poor, food source compared with an artificially enriched one. The use of cultured algae in feeding experiments can therefore overestimate the natural energetic costs of filtration as well as costs associated with the digestion and assimilation of food.

4.) Sub-optimal ventilation was associated with the feeding current during clearance of low densities of algal cells and detritus. The feeding current at moderate algae concentrations provided above-optimal ventilation.

5.) The considerable protein catabolism indicated during nutritional stress supported an earlier contention, based on gross biochemical changes, that *D. serra* relies predominantly on protein as an energy reserve.

6.) Although anaerobic respiration has not been quantified in this study or any other concerned with *D. serra*, there is indirect evidence that anaerobiosis can be a considerable component of overall metabolism. Anaerobiosis is most likely to occur during tidal exposure and locomotory activities such as burrowing and repositioning in the surf.

CHAPTER SIX

SCOPE FOR GROWTH AND REPRODUCTION

INTRODUCTION

It has become common practice to estimate the energy available for both somatic and reproductive growth, or scope for growth (SFG) in bivalves by balancing measures of energy input (ingestion X absorption) against those of energy expenditure, estimated from measures of respiration and excretion. Such budgeting is most frequently expressed in energy terms, although the importance of elemental (carbon and nitrogen) balancing has recently been recognised (Seiderer *et al.*, 1984; Seiderer & Newell, 1985; Hawkins & Bayne, 1985, Lucas *et al.*, 1987; Matthews *et al.*, 1989). This approach to estimating growth has been favoured by researchers because it provides a rapid laboratory-based assessment of energetic status and hence growth and reproductive output. Furthermore, SFG can be used to provide a sensitive and immediate index of the effects of environmental parameters such as temperature and food availability on short-term growth rates (Chapter 7).

However, there is a very important assumption involved in this type of energy budgeting. It is presumed that the quantity and quality of laboratory food mimic that which is available in the field, so that after balancing food assimilation against energy expenditure, SFG realistically reflects natural growth. This assumption has been tested by measuring actual shell growth in laboratory experiments designed to estimate SFG (Kiorboe *et al.*, 1981; Mohlenberg & Kiorboe, 1981; Riisgard & Randlov, 1981). More germane to

the study of bivalve growth in natural environments have been studies comparing SFG with ecological measures of production in the field (Griffiths & King, 1979a, b; Navarro & Winter, 1982; Hummel, 1985). In both instances, the validity of SFG as an index of natural growth has been shown to depend on the quality of food used in the laboratory.

The two previous chapters in this thesis have shown that food quality, as well as quantity, have a profound effect on rates of ingestion (IR), absorption (Ab), egestion (F), respiration (R) and excretion (U) in *D. serra*. In this chapter short-term production rates on diets of cultured algae and natural detritus are estimated by balancing rates of energy acquisition against those of expenditure (SFG = IR - [F + R + U]). The validity of using algae and naturally-derived detritus as laboratory foods was tested by comparing SFG values with field estimates of somatic and reproductive growth calculated from data collected during a 5-year ecological study of the Ouskip population (Cook & Birkett, 1984, 1986). This comparison enabled a distinction to be made between tissue and gamete production in different sized *D. serra*. Furthermore, the reliability of SFG as a true index of the condition of individuals could be established with respect to the interpretation of effects of temperature and chlorine (Chapter 7).

METHODS

ENERGY BALANCE

To calculate the energy available for growth and reproduction in *D. serra*, rates of ingestion, defaecation (Chapter 4), ammonia excretion and aerobic respiration (Chapter 5) were converted to energy equivalents (Joules) and integrated by means of the basic energy equation of Winberg (1956) [see below]. Energy values for respiration and excretion were obtained using the conversion ratios 1 ml O_2 = 20.080 J (Gnaiger, 1983) and 1 mg NH_4-N = 24.870 J (Elliot & Davison, 1975). Energy conversions relating to ingested rations of algae and detritus are given in Chapter 4.

The scope for growth and reproduction (SFG) was derived from the standard energy budget equations:

$$C = P + U + F + R,$$

$$Ab = C - F = P + R + U,$$

$$SFG = P = Ab - (R + U),$$

where C = consumption or total energy intake; P = production (the fraction of the absorbed energy used for growth and reproduction); U = absorbed energy lost in excretion; F = unabsorbed energy lost in faeces; R = energy lost as heat through respiration and Ab = energy absorbed. The relationship between these physiological rates at different ration levels and body size (g DW), was predicted from the allometric equations in Tables 4.6, 4.9, 5.1 and 5.5.

These data could also be used to calculate growth efficiencies, which are estimates of the efficiency with which food is converted into body mass (Calow, 1977). The two ratios normally calculated are gross-growth efficiency, K_1, given as P/C or SFG/C and net-growth efficiency, K_2, given as P/Ab or SFG/Ab.

ESTIMATE OF SFG FROM FIELD DATA

Relationships between different body dimensions

To facilitate easy conversion from one body dimension to another, allometric equations were calculated relating total and organic dry-tissue weight (g), shell width and length (mm) and total and organic dry-shell weight (g). Possible seasonal variability in these parameters was considered by using two sets of data from the ecological project, one obtained on 1/7/1985 (winter) and the other on 10/12/1985 (summer). This corresponds to bi-annual sampling sessions in which the intertidal zone was extensively sampled from extreme low water springs to high water. The data used in regressional analyses therefore adequately represent the size range of the *D. serra* population at Ouskip.

Growth rates

Ecological data were available as monthly plots of size-frequency distributions in which 10 growth cohorts were identified from 1981 - 1985. Changes in the position of these cohort peaks over a period of 1742 days provided a

measure of the mean growth rate of shell width within each cohort. A summary of growth data, based on 21 modal size classes ranging from 2.5 to 57.5 mm, was kindly provided by D. Birkett (University of Cape Town. pers. comm.).

These data, together with further data in Cook & Birkett (1984), were re-analysed and then fitted to Gompertz growth equations (Ricker, 1975; Bayne & Worrall, 1980) in which shell width (mm) was regressed against time in days. Growth in terms of dry-tissue and shell weight was obtained from allometric equations relating these body dimensions to shell width.

Increments in dry-tissue weight were transformed to energy values using the conversion 1 mg DW = 17.335 J (see Chapter 4). To obtain the energy content of the shell, known weights of shell fragments from different sized *D. serra* were placed in 13 N HCl for 3 days to dissolve the calcium carbonate (Griffiths & King, 1979b). Remaining matrices were rinsed in distilled water, dried and combusted in a calorimeter. This yielded a mean value of 1.203 J mg dry shell weight^{-1} (n = 6; SD = $\pm$0.212).

Gonad Output

As part of the ecological project, 20 - 30 adult *D. serra* (30 - 57 mm shell width) were collected monthly to determine breeding cycles and the relationship between shell width and wet or dry tissue weight. For the purposes of the present study, these data, spanning a period of 4 years (1982 - 1985), were fitted to monthly regressions in which WW =

$a \cdot SW^b$, where WW = wet-tissue weight (g) and SW = shell width (mm). The dry-weight data were insufficient for regressional analysis, but proved useful for assessing wet to dry weight ratios for each month.

Temporal changes in the wet weight of a standard sized individual (45 mm) were determined from these monthly allometric equations and a decline in weight was assumed to represent the release of gametes. Regression equations were then used to calculate the body weight of standard sized bivalves before and after the identified spawning events. Naturally, weight changes would incorporate growth increments simultaneously with reproductive losses and this would result in an underestimation of P_r. Accordingly, adjustments for growth over periods of weight loss were made using the growth-rate equation.

To obtain an energy equivalent of P_r (kJ yr^{-1}), samples of ripe gonad were carefully extricated from the visceral mass of 20 bivalves, oven-dried (60^0C for 3 days) and then combusted in a microbomb calorimeter. The average energy content of 1 mg dried gonad tissue was 19.101 J (n = 6; SD = $\pm$ 1.211). This factor was used to transform weight loss to energy values following wet- to dry-weight conversion.

RESULTS

ENERGY BUDGETS

The interactive effects of body size and diet on energy acquisition and expenditure are presented in Table 6.1 for an algal diet and Table 6.2 for one of detritus. The budgets, expressed in J hr^{-1}, identify the rates of energy ingestion and absorption, energy loss in the form of respiration, defaecation and ammonia-N excretion and finally, the subsequent energy profit or loss available for growth and reproduction. Regression equations for these various physiological rates, calculated from the values in Tables 6.1 and 6.2, are summarised in Tables 6.3 (algae) and 6.4 (detritus).

Two major factors emerge from these data with respect to dietary quality. Firstly, scope for growth and reproduction (SFG) with algae as a food resource vastly exceeded those of detritus at all comparable ration levels and body sizes. This contrast arises from the much higher ingestion rates and absorption efficiencies on an algal diet (see Chapter 4). These result in maximum SFG being 53 times greater in a 1-g animal fed algae compared with detritus. Secondly, SFG was positive over a much wider range of algal rations, an energy deficit only being evident at concentrations <2.5 mg DW l^{-1}. With detritus, on the other hand, bivalves lost weight at all rations except those between 5.5 and 8.1 mg DW l^{-1}.

Table 6.1. ENERGY BUDGET - ALGAL DIET. Energy ingested and its use in the different physiological processes of *D. serra* in relation to body size and seven concentrations of *T. suecica* at 15°C . The following conversion factors were used to obtain energy values:
17.336 J mg dry tissue weight $^{-1}$; 20.700 J mg dry algal weight $^{-1}$; 20.080 J ml O_2^{-1} (Gnaiger, 1983); 24.870 J mg NH_4 - N^{-1} (Elliot & Davison, 1975).

RATION	BODY SIZE			ENERGY INGESTED		ENERGY ABSORBED	METABOLISM			ENERGY FOR GROWTH & REPRODUCTION
X 10^6 l^{-1} mg DW l^{-1}	Dry tissue weight (g)	Shell width (mm)	Tissue energy (kJ)	Dry weight (mg h^{-1})	Energy content (J h^{-1})	(J h^{-1})	Energy lost in faeces (J h^{-1})	Energy used in respiration (J h^{-1})	Energy lost in excretion (J h^{-1})	(J h^{-1})
5 1.245	0.10	14.04	1.74	0.065	1.346	0.498	0.848	1.526	0.229	-1.257
	0.50	24.19	8.67	0.133	2.753	1.019	1.734	4.973	0.564	-4.518
	1.00	30.57	17.34	0.180	3.726	1.379	2.347	8.271	0.831	-7.723
	3.00	44.32	52.01	0.292	6.042	2.236	3.806	18.305	1.538	-17.607
	5.00	52.67	86.68	0.365	7.564	2.799	4.765	26.954	2.047	-26.202
10 2.490	0.10	14.04	1.74	0.265	5.493	3.043	2.450	1.526	0.229	1.288
	0.50	24.19	8.67	0.584	12.086	8.696	5.390	4.973	0.564	1.159
	1.00	30.57	17.34	0.820	16.974	9.404	7.570	8.271	0.831	0.302
	3.00	44.32	52.01	1.405	29.080	16.110	12.970	18.305	1.538	-3.733
	5.00	52.67	86.68	1.804	37.349	20.691	16.658	26.954	2.047	-8.310
15 3.735	0.10	14.04	1.74	0.589	12.201	6.723	5.478	1.526	0.229	4.968
	0.50	24.19	8.67	1.452	30.047	16.556	13.491	4.973	0.564	11.019
	1.00	30.57	17.34	2.140	44.298	24.408	19.889	8.271	0.831	15.306
	3.00	44.32	52.01	3.959	81.954	45.157	36.797	18.305	1.538	25.314
	5.00	52.67	86.68	5.270	109.096	60.112	48.984	26.954	2.047	34.111
25 6.225	0.10	14.04	1.74	1.225	25.358	15.215	10.144	1.607	0.229	13.379
	0.50	24.19	8.67	3.065	63.446	38.067	25.379	5.452	0.564	32.051
	1.00	30.57	17.34	4.550	94.185	56.511	37.674	9.227	0.831	46.453
	3.00	44.32	52.01	8.511	176.174	105.704	70.470	20.967	1.538	83.179
	5.00	52.67	86.68	11.387	235.711	141.427	94.284	31.301	2.047	108.079
30 7.470	0.10	14.04	1.74	1.772	35.643	25.235	10.408	1.778	0.229	23.228
	0.50	24.19	8.67	3.728	77.175	54.640	22.535	5.655	0.564	48.421
	1.00	30.57	17.34	5.200	107.640	76.209	31.431	9.308	0.831	66.070
	3.00	44.32	52.01	8.811	182.386	129.129	53.257	20.262	1.538	107.329
	5.00	52.67	86.68	11.259	232.066	165.109	68.055	29.608	2.047	133.454
35 8.715	0.10	14.04	1.74	1.170	24.219	17.970	6.249	1.778	0.229	15.963
	0.50	24.19	8.67	2.616	54.151	40.180	13.971	5.655	0.564	33.961
	1.00	30.57	17.34	3.700	76.590	56.829	19.761	9.308	0.831	46.690
	3.00	44.32	52.01	6.409	132.658	98.432	34.226	20.262	1.538	76.632
	5.00	52.67	86.68	8.273	171.260	127.075	44.185	29.608	2.047	95.420
40 9.960	0.10	14.04	1.74	0.445	9.218	5.697	3.521	1.778	0.229	3.690
	0.50	24.19	8.67	1.079	22.339	13.805	8.534	5.655	0.564	7.586
	1.00	30.57	17.34	1.580	32.706	20.212	12.494	9.308	0.831	10.073
	3.00	44.32	52.01	2.891	59.847	36.986	22.861	20.262	1.538	15.186
	5.00	52.67	86.68	3.829	79.261	48.983	30.278	29.608	2.047	17.328

Table 6.2. ENERGY BUDGET - DETRITAL DIET. Energy ingested and its use in the different physiological processes of *D. serra* in relation to body size and five concentrations of seafoam detrital particles at 15 °C . The following conversion factors were used to obtain energy values:
17.336 J mg dry tissue weight $^{-1}$; 9.909 J mg dry detrital weight $^{-1}$; 20.080 J ml O_2^{-1} (Gnaiger, 1983); 24.870 J mg NH_4 - N^{-1} (Elliot & Davison, 1975).

RATION	BODY SIZE			ENERGY INGESTED		ENERGY ABSORBED	METABOLISM			ENERGY FOR GROWTH & REPRODUCTION
X 10^6 l^{-1} mg DW l^{-1}	Dry tissue weight (g)	Shell width (mm)	Tissue energy (kJ)	Dry weight (mg h^{-1})	Energy content (J h^{-1})	(J h^{-1})	Energy lost in faeces (J h^{-1})	Energy used in respiration (J h^{-1})	Energy lost in excretion (J h^{-1})	(J h^{-1})
5 1.365	0.10	14.04	1.74	0.076	0.753	0.429	0.324	1.398	0.239	-1.208
	0.50	24.19	8.67	0.160	1.585	0.903	0.682	3.854	0.543	-3.494
	1.00	30.57	17.34	0.220	2.179	1.242	0.937	5.964	0.774	-5.498
	3.00	44.32	52.01	0.365	3.614	2.060	1.554	11.915	1.355	-11.210
	5.00	52.67	86.68	0.461	4.568	2.604	1.964	16.439	1.759	-15.594
10 2.730	0.10	14.04	1.74	0.192	1.903	0.932	0.971	1.398	0.239	-0.705
	0.50	24.19	8.67	0.435	4.310	2.112	2.198	3.854	0.543	-2.285
	1.00	30.57	17.34	0.620	6.144	3.011	3.133	5.964	0.774	-3.727
	3.00	44.32	52.01	1.086	10.758	5.272	5.486	11.915	1.355	-7.998
	5.00	52.67	86.68	1.409	13.962	6.841	7.121	16.438	1.759	-11.357
20 5.460	0.10	14.04	1.74	0.379	3.756	1.916	1.840	1.398	0.239	0.279
	0.50	24.19	8.67	1.028	10.186	5.195	4.991	3.854	0.543	0.798
	1.00	30.57	17.34	1.580	15.656	7.985	7.671	5.964	0.774	1.247
	3.00	44.32	52.01	3.122	30.939	15.779	15.160	11.915	1.355	2.509
	5.00	52.67	86.68	4.286	42.470	21.660	20.810	16.439	1.759	3.462
30 8.190	0.10	14.04	1.74	0.525	5.202	2.029	3.173	1.398	0.239	0.392
	0.50	24.19	8.67	1.174	11.633	4.537	7.096	3.854	0.543	0.140
	1.00	30.57	17.34	1.660	16.449	6.415	10.034	5.964	0.774	-0.323
	3.00	44.32	52.01	2.875	28.488	11.110	14.192	11.915	1.355	-2.160
	5.00	52.67	86.68	3.712	36.782	14.345	22.437	16.439	1.759	-3.853
40 10.920	0.10	14.04	1.74	0.404	4.003	0.681	3.322	1.398	0.239	-0.096
	0.50	24.19	8.67	0.949	9.404	1.599	7.805	3.834	0.543	-2.798
	1.00	30.57	17.34	1.370	13.575	2.308	11.267	5.964	0.774	-4.430
	3.00	44.32	52.01	2.452	24.301	4.131	20.169	11.915	1.355	-9.139
	5.00	52.67	86.68	3.215	31.857	5.416	26.441	16.439	1.759	-12.782

Table 6.3. ALGAL DIET - a - and b - values calculated for different physiological processes (J h^{-1}) in relation to body size (g DW) and seven concentrations of *T. suecica*. Regressions take the form of Physiological rate = a (Body size)^{b} and have been calculated from values presented in Table 6.1. a = intercept; b = slope.

PHYSIOLOGICAL PROCESS	ALGAL CONCENTRATION													
	X 10^{6} cells l^{-1} / mg DW l^{-1}	5 / 1.25		10 / 2.49		15 / 3.34		25 / 6.23		30 / 7.47		35 / 8.72		40 / 9.96	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b
Energy ingested		3.72	0.44	16.97	0.49	44.30	0.56	94.19	0.57	107.64	0.48	76.59	0.50	32.71	0.55
Energy absorbed		1.38	0.44	9.40	0.49	24.41	0.56	56.51	0.57	76.22	0.48	56.83	0.50	20.21	0.55
Energy lost in faeces		2.35	0.44	7.57	0.49	19.89	0.56	37.68	0.57	31.43	0.48	19.76	0.50	12.49	0.55
Energy used in respiration		8.25	0.73	8.25	0.73	8.25	0.73	9.20	0.79	9.29	0.72	9.29	0.72	9.29	0.72
Energy lost in excretion		0.83	0.56	0.83	0.56	0.83	0.56	0.83	0.56	0.83	0.56	0.83	0.56	0.83	0.56
Energy for growth & reproduction		----	----	----	----	15.28	0.49	46.13	0.54	65.55	0.45	46.23	0.46	9.64	0.40

Table 6.4. DETRITAL DIET - a - and b - values calculated for different physiological processes (J h^{-1}) in relation to body size (g DW) and five concentrations of seafoam detrital particles. Regressions take the form of Physiological rate = a (Body size)^{b} and have been calculated from values presented in Table 6.2. a = intercept; b = slope.

PHYSIOLOGICAL PROCESS	X 10^{6} l^{-1} mg DW l^{-1}	DETRITAL CONCENTRATION									
		5 1.37		10 2.73		20 5.46		30 8.19		40 10.92	
		a	b	a	b	a	b	a	b	a	b
Energy ingested		2.18	0.46	6.15	0.51	15.66	0.62	16.45	0.50	13.57	0.53
Energy absorbed		1.24	0.46	3.01	0.51	7.99	0.62	6.42	0.50	2.31	0.53
Energy lost in faeces		0.94	0.46	3.13	0.51	7.67	0.62	9.62	0.50	11.27	0.53
Energy used in respiration		5.96	0.63	5.96	0.63	5.96	0.63	5.96	0.63	5.96	0.63
Energy lost in excretion		0.77	0.51	0.77	0.51	0.77	0.51	0.77	0.51	0.77	0.51
Energy for growth & reproduction		----	----	----	----	1.24	0.64	----	----	----	----

SFG increased with increasing algal concentrations, reaching a peak at 7.5 mg DW l^{-1} but declining with a further increase in ration to 10.0 mg l^{-1}. This reversal of energy balance follows the drastic fall in absorption efficiencies at high rations concomitant with a reduction in ingestion. Budgeting at food levels >10 mg algae l^{-1} was not possible in this study because ingestion could not be accurately quantified in the face of copious pseudofaeces production. Relatively, the best SFG on a detrital diet was only attained between 5 and 8 mg DW l^{-1}.

Proportional amounts of energy used or lost in each of physiological process are presented in Fig. 6.1 and 6.2 for algal and detrital diets respectively. At low algal rations, energy losses were principally attributable to respiration, followed by defaecation. As ration increased, a greater energetic fraction was lost in the egestion of faeces. Egestion, rather than respiration, then became the principle determinant of the proportion of ingested energy available for growth and reproduction. Beyond the optimum algal ration (7.5 mg l^{-1}), energy lost in faeces diminished so that at 10 mg l^{-1}, energy loss was proportionally the same for respiration and egestion. The drastic decline in SFG at high algal rations is clearly depicted in Fig. 6.1.

At low detrital concentrations, most ingested energy was also channelled into respiration (Fig. 6.2). At 5.5 mg l^{-1}, the only ration at which energy balance was positive for all sizes of *D. serra*, energy utilisation was equally

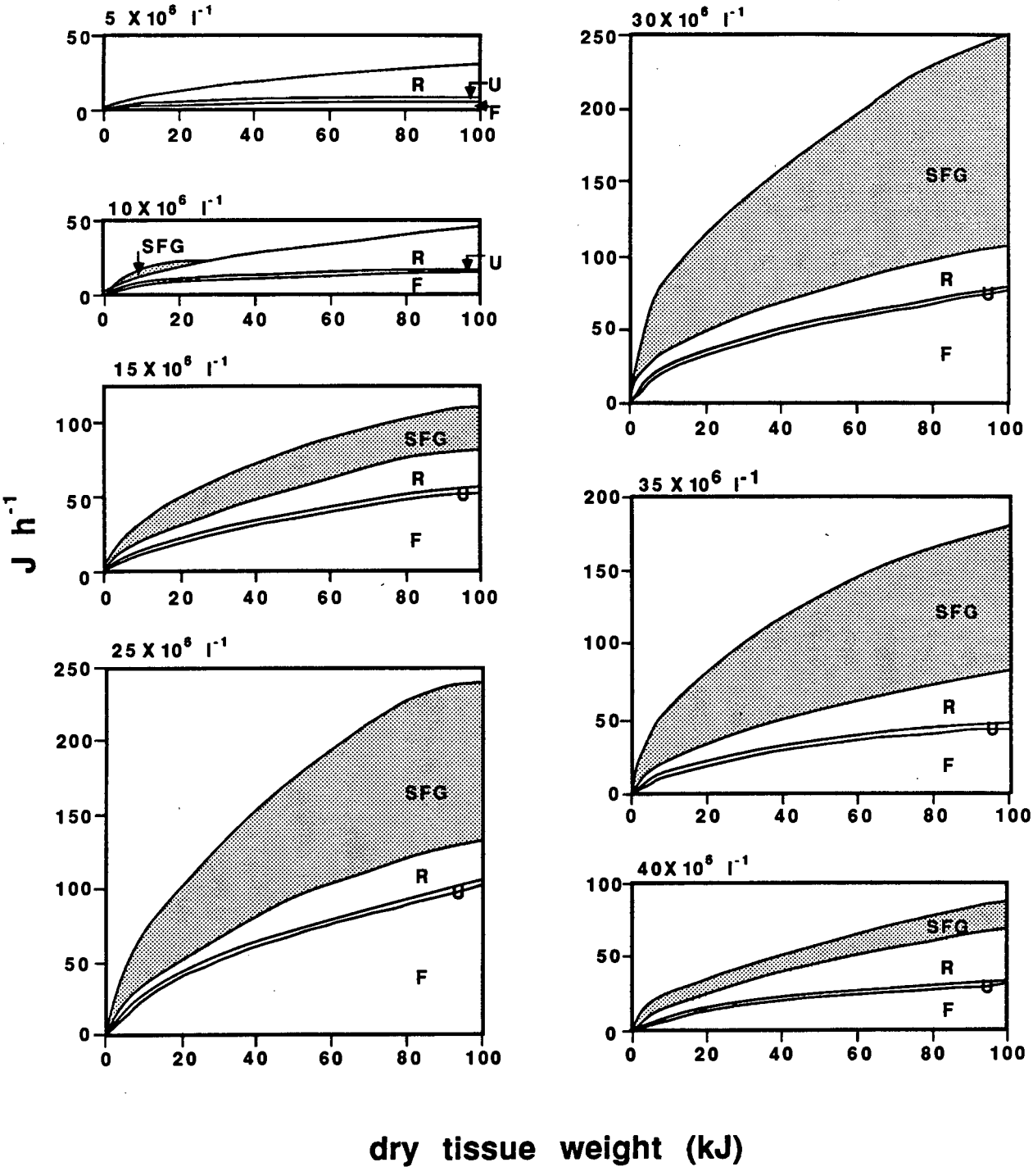

Fig. 6.1. ALGAL DIET: Size-related energy partitioning between respiration (R), defaecation (F) and excretion (U) at different ration levels of *T. suecica*. Shaded areas indicate energy available for growth and reproduction (SFG).

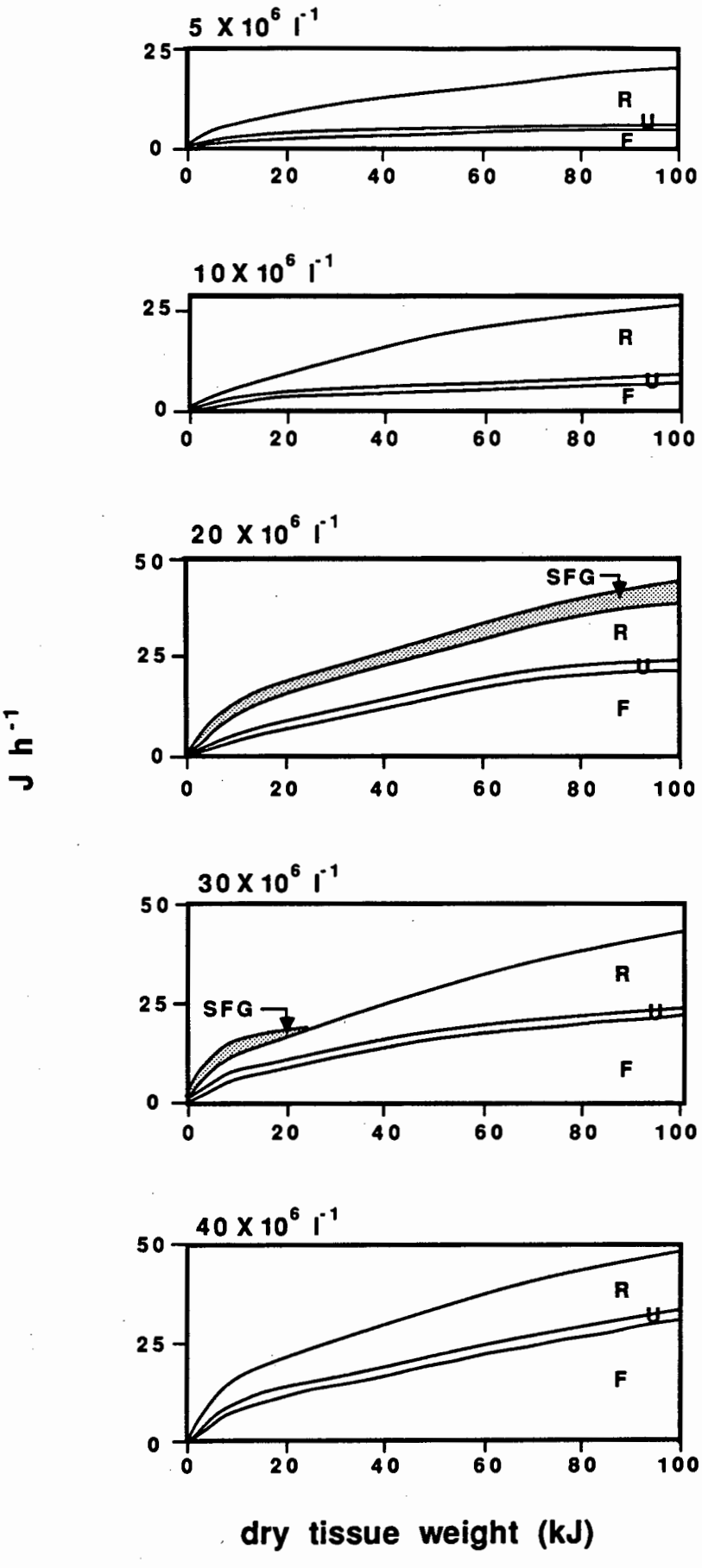

Fig. 6.2. DETRITAL DIET: Size-related energy partitioning between respiration (R), defaecation (F) and excretion (U) at different ration levels of seafoam detritus. The shaded areas indicate energy available for growth and reproduction (SFG).

partitioned between R and F. At higher concentrations, however, defaecation became the principle source of energy loss. Both Figs. 6.1 and 6.2 show that, irrespective of dietary quality, quantity or animal size, losses via ammonia excretion were negligible.

Since it is often more meaningful to view the relation between body size and physiological rates in weight-specific terms, SFG as percentage change in body energy day^{-1} has been plotted against ration level for 3 sizes of *D. serra* (Fig. 6.3). Relative growth rates of small bivalves (0.1 g) were markedly faster than those of individuals of 1 and 5 g, especially when fed algae. Maximum percentage change with algae as food was 30% in small animals compared with 5% in the larger ones. Ingesting detritus resulted in a corresponding size-related change of only 0.5% and 0.1%.

The optimum algal ration for maximum daily change in body energy for all sizes occurred at 7.5 mg l^{-1}, i.e. the same ration for optimum absolute growth. With detritus as food, optimum ration was a function of size, maximum weight-specific growth for 0.1-g individuals occurring at a higher concentration (8.2 mg l^{-1}) than for 5-g ones (5.5 mg l^{-1}).

On both diets the maintenance ration, that is the point of zero growth, was lowest for small sizes approximating 2 mg algae and 4 mg detritus l^{-1}. Larger animals required 3 mg algae and about 5 mg detritus l^{-1} to meet the basic demands of daily maintenance.

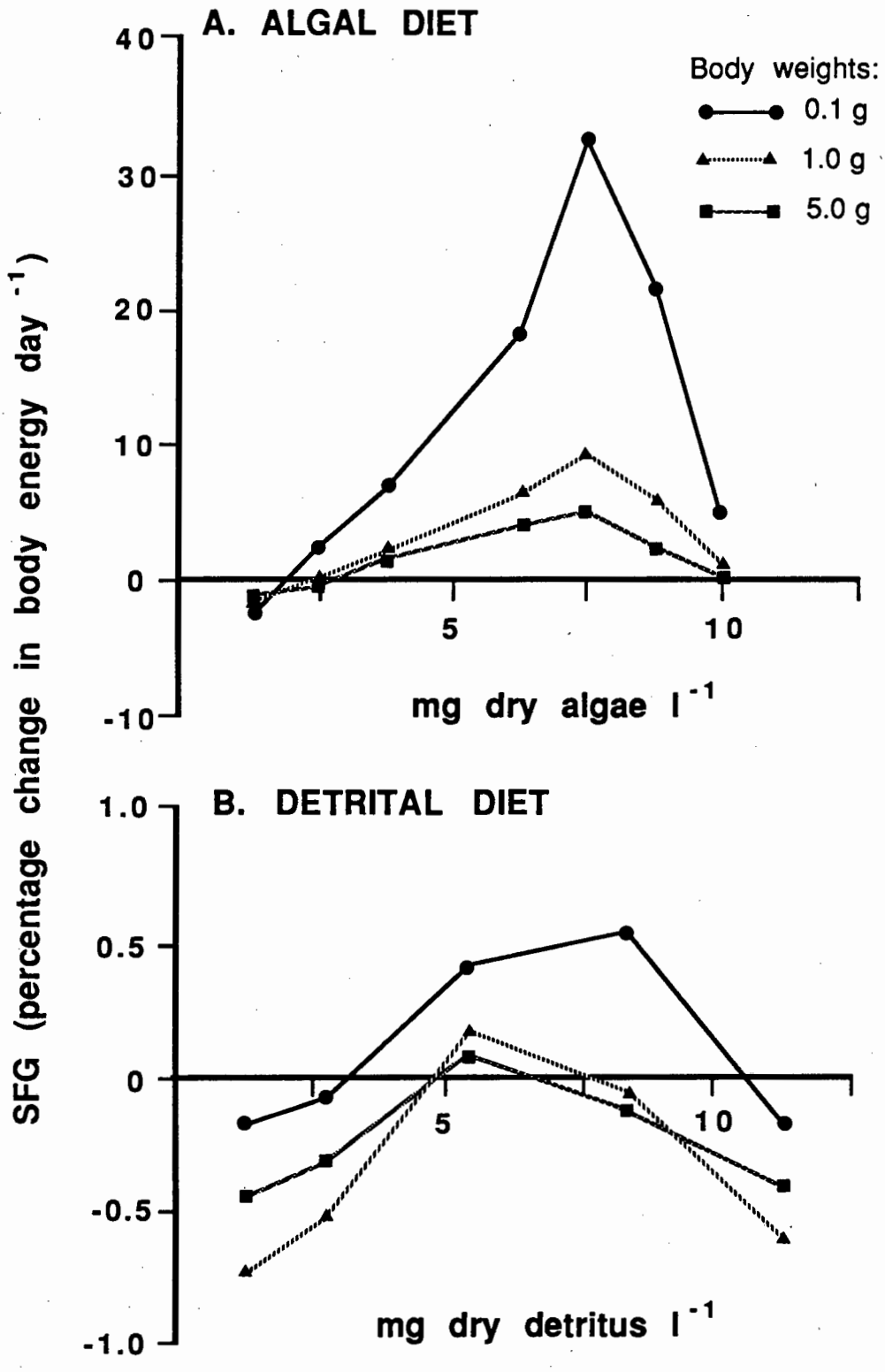

Fig. 6.3. SFG as percentage change in body energy per day for animals weighing 0.1, 1.0 and 5.0 g in relation to available rations (mg DW l^{-1}) of algae (A) and detritus (B).

It is also obvious from Fig. 6.3 that 0.1 g animals ingesting detritus, maintained positive growth over a much wider range of rations than larger ones. Such utilisation of a resource, even when poor in quality, provides an ecological advantage to juveniles which need to grow rapidly once settled as spat. In contrast, medium- to large- sized *D .serra* were unable to maintain positive daily energy gain at most detrital concentrations investigated.

GROWTH EFFICIENCIES

The efficiency with which food was converted into body mass was expressed in terms of gross growth efficiency (K_1), or growth per unit ingested ration, and net growth efficiency (K_2), equivalent to growth per unit absorbed ration. These data are plotted against rates of ingestion and absorption in Fig. 6.4 for three body sizes.

The smallest bivalves (0.1 g DW) demonstrated the highest K_1 and K_2 values irrespective of diet or rates of food ingestion and absorption. Efficiencies when fed algae reached asymptotic size-related values of 50 - 70% for K_1 and 80 - 90% for K_2. In detritus-feeding animals, efficiencies were much lower ranging from 3 - 8% for K_1 and 8 - 18% for K_2. On this diet, asymptotic efficiencies were not demonstrated by the two smaller sizes (0.1 & 1.0 g), but peak values around 8 - 10% were reached by 5.0 g animals. Juveniles in their most active growing phase while feeding

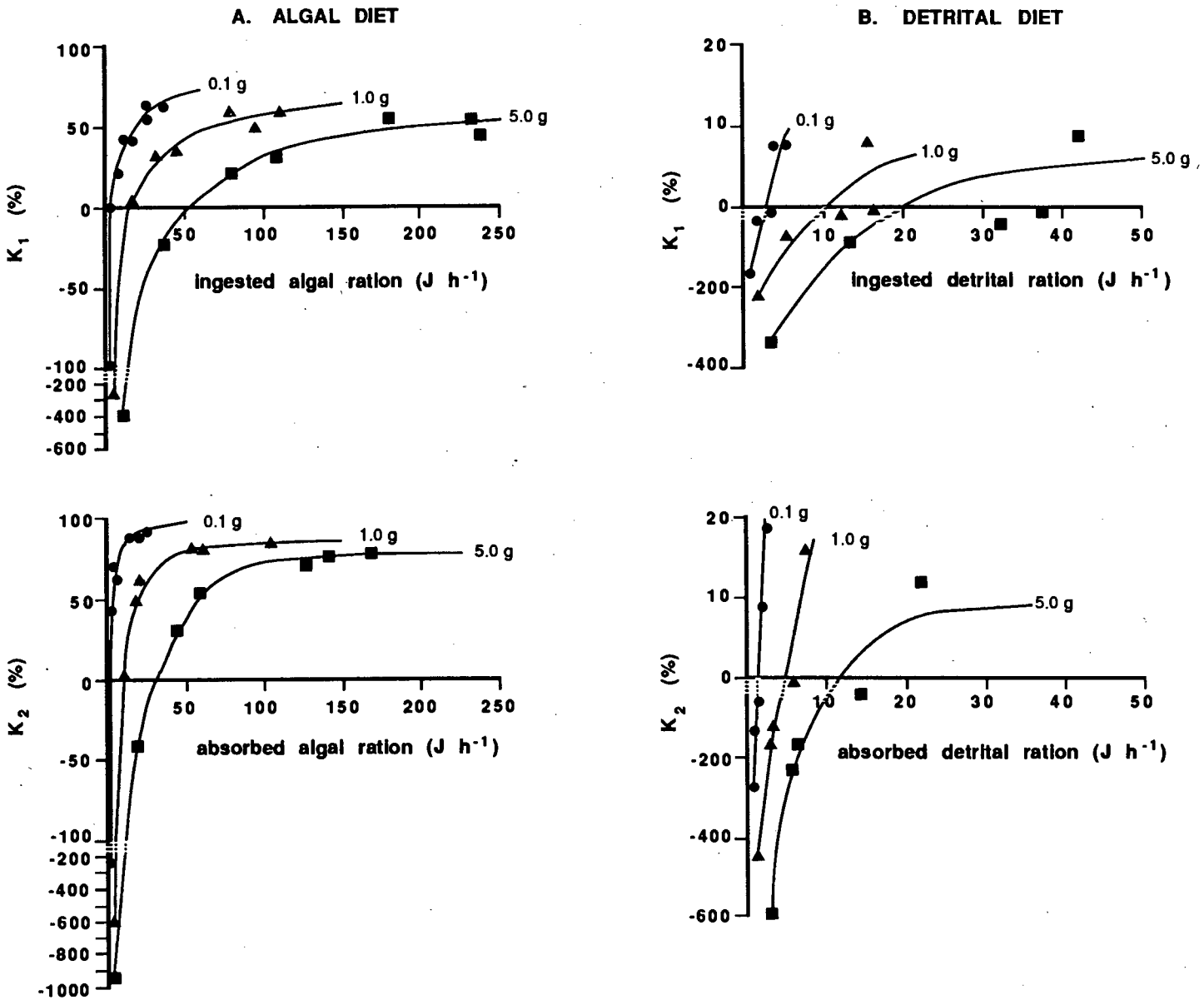

Fig. 6.4. Gross (K_1) and net (K_2) growth efficiencies as a function of ingested and absorbed rations of algae (A) and detritus (B) in bivalves of 0.1, 1.0 and 5.0 g dry weight. K_1 = SFG/IR & K_2 = SFG/AR based on data in Tables 6.1 & 6.2.

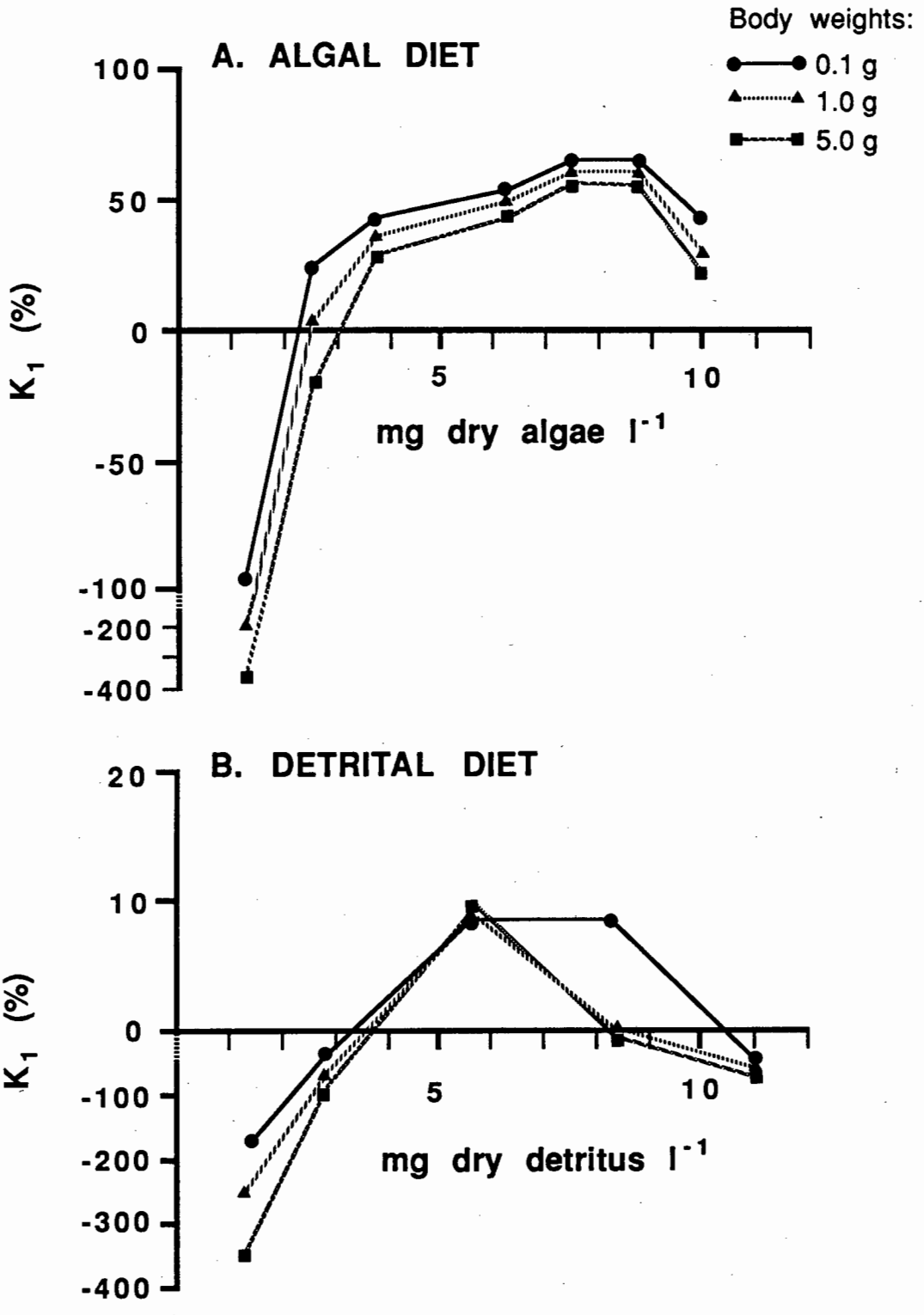

Fig. 6.5. Gross growth efficiencies (K_1) at different concentrations of algae (A) and detritus (B) [mg DW l^{-1}] in animals of 0.1, 1.0 and 5.0 g dry weight.

FIELD ESTIMATES OF SFG

Body dimensions

The relationships between dry tissue weight, shell width and a number of other body dimensions are summarised in Table 6.5 in the form of allometric equations. All relations were highly significant ($P < 0.01$) as indicated by the Pearson product-moment correlation coefficients (r) and small standard errors. To ascertain whether there was any seasonal variability in these interrelationships, equations were initially derived from two sets of data, one from 1/7/1985 (winter) and the other from 10/12/1985 (summer). Since Student-*t* tests ($t_{0.01(2)}$; DF = 266) indicated no significant difference between slopes or intercepts, the two sets of data were combined in the calculation of equations in Table 6.5. Regressions were principally used to predict dry tissue weight (g) from shell width (mm) and vica versa.

Growth rates (P_g)

Growth data were best fitted to a Gompertz equation (r = 0.98) rather than von Bertalanffy (r = 0.94) or logistic (r = 0.92) curves. The resultant plot of shell width (mm) versus age in days is illustrated in Fig. 6.6 and described by the following equation:

$$SW_t = 59.138 \cdot e^{-e(0.939 - 0.002t)},$$

where SW = shell width and t = days.

Table 6.5. Relationships between a variety of body dimensions of *D. serra*. Dry tissue weight (DW; g) is regressed on the independent variables, organic dry tissue weight (ODW; g), shell width (SW) and length (SL; mm), and on shell dry weight (SDW) and organic dry weight (SODW; g). Furthermore, SW is regressed as the dependent variable on DW, ODW, SL, SDW and SODW. n = 267 for all regressions of the form (dependent variable) = a·(independent variable)^{b}.

Dependent Variable	a intercept	Independent Variable	b slope	r	SE
DW	1.170	ODW	0.959	0.988	0.062
DW	5.24×10^{-5}	SW	2.875	0.986	0.069
DW	0.43×10^{-5}	SL	3.206	0.985	0.070
DW	0.192	SDW	0.901	0.983	0.072
DW	4.204	SODW	0.624	0.968	0.103
SW	30.573	DW	0.338	0.986	0.024
SW	32.299	ODW	0.326	0.980	0.028
SW	0.425	SL	1.113	0.997	0.010
SW	17.360	SDW	0.313	0.997	0.012
SW	50.223	SODW	0.302	0.968	0.035

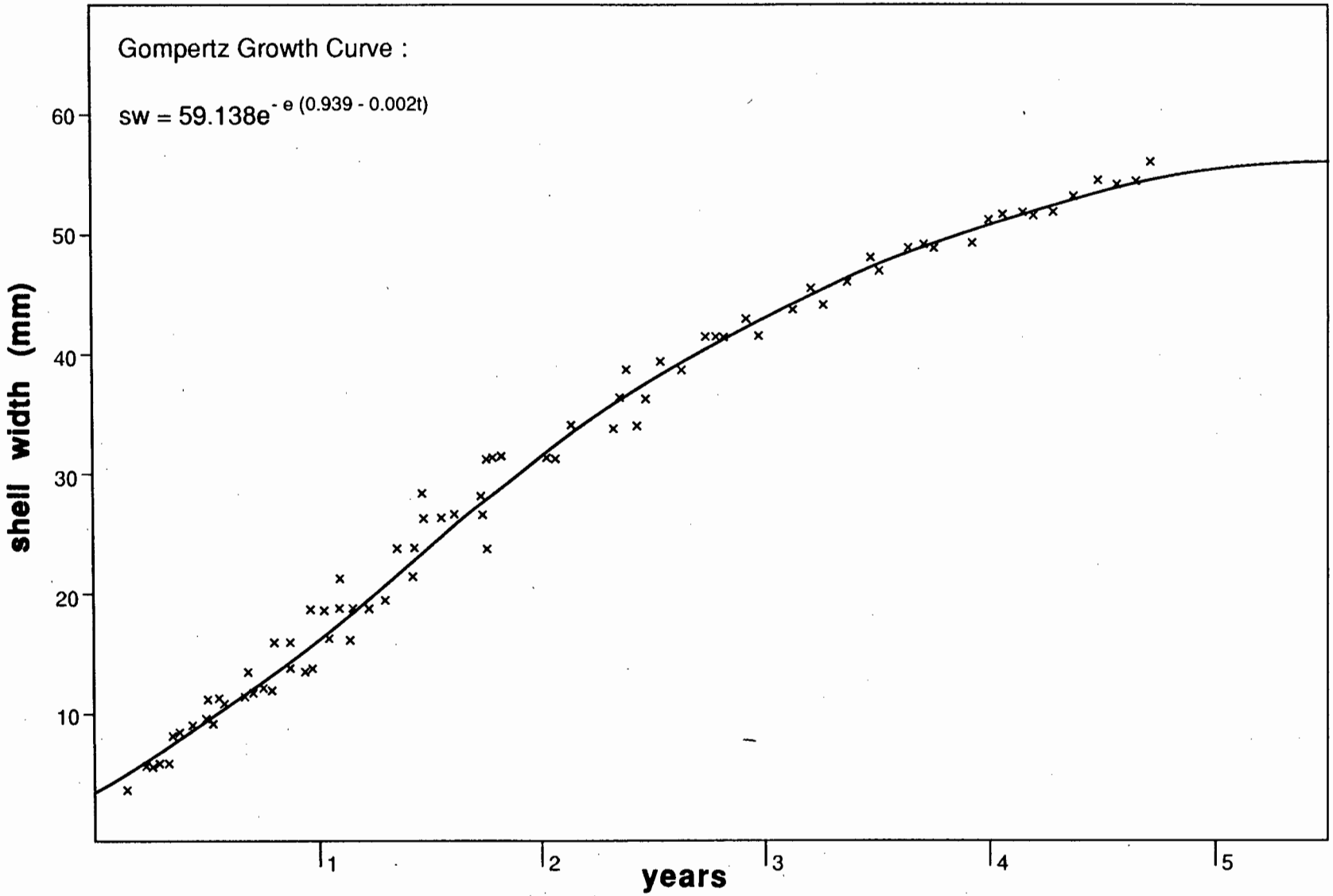

Fig. 6.6. Gompertz growth curve for the *D. serra* population at Ouskip based on data from 1981 to 1985. In the growth equation, SW = shell width (mm) and t = time (years). n = 80, r = 0.98.

With each successive year, growth rate slowed towards an asymptote after 5 to 6 years. Newly settled spat in the intertidal were approximately 4 mm in width corresponding to 2.80 mg dry tissue weight and 11.72 mg with the shell. After 1 year, shell width increased to 17 mm, after 2 years, 32 mm and after 3 and 4 years, 44 and 52 mm respectively. A maximum shell width of approximately 55 mm was attained after 5 - 5.5 yrs. This is equivalent to a dry-tissue weight of 5.28 g and for the whole animal (shell + tissue) it is 41.59 g.

Reproductive output (P_r)

The monthly regressions used to identify reproductive events are summarised in Table 6.6 for the years 1982 -1985. From these regressions the monthly wet-tissue weights of a standard 45-mm animal were calculated and plotted against time (months) to identify periods of weight loss (Fig. 6.7). Such periods or "spawning events" were inconsistent in number and timing from year to year, varying from 2 in 1982 and 1983, to 4 in 1984 and 1 in 1985.

Wet-weight loss in relation to shell width was calculated from the allometric regressions and converted to dry weight using mean-monthly wet:dry weight ratios, which ranged from about 4 to 6. After taking account of tissue growth (Fig. 6.6), dry weight was converted to energy equivalents which in turn were summed for each year to arrive at P_r in kJ yr^{-1}. These data are plotted against shell width in Fig. 6.8 for each year and as an average over

Table 6.6. Monthly regressions of wet-tissue weight (WW; g) versus shell width (SW; mm) where $WW = a \cdot SW^b$. These regressions identified temporal weight changes which were subsequently used to quantify reproductive output between 1982 and 1985.

YEAR	MONTH	DAY	a X 10^{-3}	b	r	n
1982	2	11	4.997	2.093	0.947	20
	4	23	14.600	1.818	0.982	17
	5	25	9.600	1.968	0.935	20
	6	24	5.630	2.097	0.939	22
	8	6	14.680	1.837	0.966	20
	9	17	14.350	1.902	0.899	20
	11	3	10.197	1.966	0.930	20
	11	17	22.890	1.729	0.843	28
	12	1	9.250	2.005	0.968	20
1983	1	5	9.900	1.991	0.971	20
	2	14	1.500	2.437	0.975	20
	3	16	3.200	2.223	0.964	20
	4	14	3.000	2.237	0.900	20
	7	21	2.100	2.324	0.993	20
	8	9	4.800	2.132	0.948	20
	10	20	1.100	2.504	0.884	20
	11	21	0.200	2.934	0.880	20
1984	1	5	1.330	2.482	0.789	20
	2	2	0.044	3.345	0.696	20
	3	19	4.400	2.197	0.846	20
	5	2	29.800	1.665	0.854	20
	6	28	0.300	2.822	0.909	20
	7	30	38.500	1.572	0.654	20
	8	27	1.100	2.477	0.746	20
	10	24	2.840	2.251	0.880	20
	12	6	14.720	1.787	0.634	20
1985	1	10	13.650	1.849	0.798	20
	2	16	6.300	2.064	0.901	20
	4	9	26.180	1.729	0.763	22
	6	4	4.330	2.158	0.606	20
	7	2	1.490	2.351	0.699	22
	8	20	2.150	2.272	0.825	25
	9	17	36.740	1.527	0.698	20
	10	16	17.040	1.146	0.658	20
	11	13	1.380	2.404	0.787	20
	12	11	20.002	1.194	0.637	20

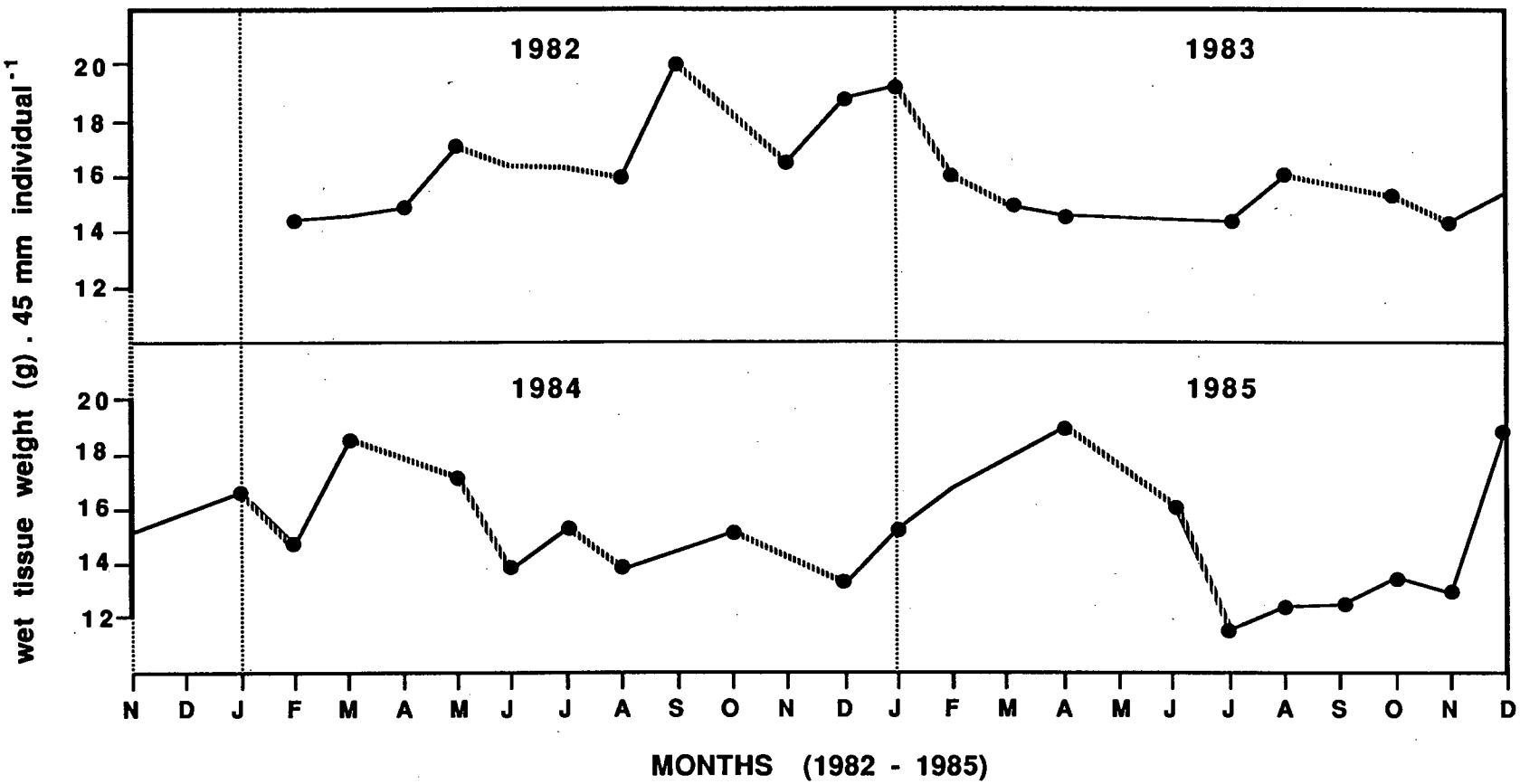

Fig. 6.7. Fluctuations in wet tissue weight of a 45-mm individual over 4 years as derived from monthly width-weight regressions (Table 6.6). Hatched lines identify periods of weight loss assumed to be synonomous with spawning.

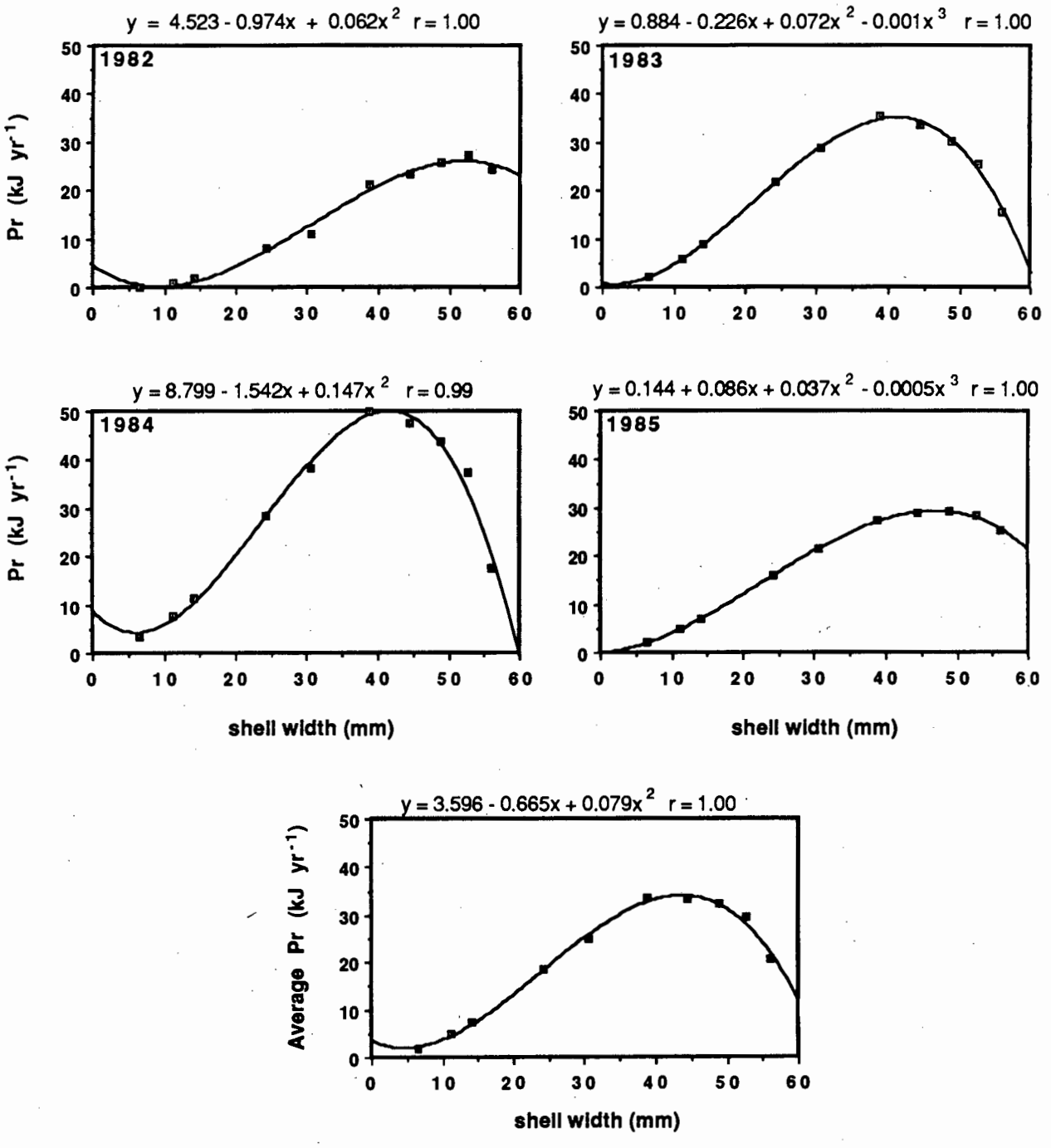

Fig. 6.8. Annual variability in the relationship between body size (shell width, mm) and reproductive output (Pr, kJ yr^{-1}) of the *D. serra* population at Ouskip from 1982 to 1985. Pr is also presented as an average over these 4 years. All data were fitted to two- or three-order polynomials.

the four years. All data were fitted to two- or three order polynomials.

Peak reproductive output, which was attained in bivalves of 40 - 45 mm, showed annual variability ranging from 25 (1982) to 50 (1984) kJ yr^{-1}. When data collected over the 4 years were averaged, a mean peak P_r of 35 kJ yr^{-1} was obtained. A consistent feature throughout the data was the rapid decline in P_r in bivalves >45 - 50 mm so that at 60 mm, reproductive output was considerably reduced.

Total production (P_g + P_r = SFG)

The amounts of energy invested in growth, reproduction and hence overall production in *D. serra* of various body sizes are presented in Table 6.7. For P_g, a distinction is made between growth with and without the shell. However, only tissue growth is considered as a component of total P to enable direct comparison with laboratory estimates of SFG, which referred to flesh alone.

Absolute tissue growth was an increasing function of body size up to a dry tissue weight of 2 g, beyond which growth declined, so that the annual P_g of a 6-g animal approximated that of one weighing only 0.01 g. In terms of growth of the whole animal, a considerable fraction was diverted into shell production. Energy contributions toward P_g (tissue + shell) increased from 26 to 35% over the size range given in Table 6.7. However, the proportion of shell growth relative to overall annual production (P_g[tissue + shell] + P_r) declined from 20 to 12% with increasing size.

Values for P_r in Table 6.7 are derived from the average annual reproductive output illustrated in Fig. 6.8 in which gonad production initially increased and then decreased again with increasing bivalve weight. Both P_r and P_g peaked in 2-g individuals resulting in a maximum total production of 62 kJ yr^{-1} $animal^{-1}$ at this size.

Reproductive effort given by (P_r) as a percentage of total tissue production (P), continued to form a progressively larger portion of P with increasing size. A 6-g bivalve, for instance, channelled 73% of its annual production into gonad material, leaving only 27% for tissue growth. However, even though reproductive effort is proportionally high in these larger bivalves, smaller but more sexually active individuals produced greater quantities of gonad material (Table 6.7).

That small bivalves (0.01 g) direct 29% of their production into reproduction is, of course, inaccurate. This error probably has its origin in the regressions of shell width to wet-tissue weight presented in Table 6.6. These equations were derived from data on adult bivalves (>30 mm) only, thereby increasing the degree of error in predicting weight changes in small bivalves. In these analyses, far greater weight change was attributed to small bivalves than could possibly have occurred. Histological sections have shown that undifferentiated gonad material first appears in *D. serra* of about 16 - 20 mm shell width, whilst differentiated gonads are never observed in those

Table 6.7. Relationship between body size (DW; g and SW; mm) and annual production (kJ yr^{-1}). DW = dry tissue weight; SW = shell width; t = time; t + 1 = time one year later; $P_g(T)$ = production as tissue growth; $P_g(T+S)$ = production as tissue and shell growth; P_r = reproductive output; P_g+P_r = P = total tissue production; P_r/P = P_r as a percentage of P; B = initial tissue biomass (J).

BODY SIZE			PRODUCTION (kJ yr^{-1})				P:B RATIOS	
DW	SW_t	$SW_{(t+1)}$	$P_g(T)$	$P_g(T+S)$	P_r	P_g+P_r	P_r/P	P:B
0.01	6.45	19.55	4.49	6.08	1.87	6.35	29.37	36.73
0.05	11.11	25.65	9.30	12.91	4.82	14.11	34.13	16.28
0.10	14.04	28.69	12.29	17.27	7.29	19.58	37.21	11.29
0.50	24.19	37.78	22.48	32.53	18.58	41.06	45.25	4.74
1.00	30.57	42.44	26.53	38.95	24.98	51.51	48.49	2.97
2.00	38.64	47.92	28.44	42.40	33.52	61.96	54.09	1.77
3.00	44.32	51.22	25.38	38.20	33.41	58.79	56.83	1.15
4.00	48.84	53.79	20.84	31.59	32.32	53.17	60.79	0.76
5.00	52.67	55.89	15.08	22.98	29.71	44.79	66.34	0.52
6.00	56.02	57.50	7.53	11.52	20.80	28.32	73.42	0.27

<24 mm (de Villiers, 1975a; D. Birkett, pers. comm.). Nevertheless, the indirect method used in this study to assess reproductive output provided a reasonable estimate of P_r in sexually mature individuals.

Annual production in the field is compared to laboratory SFG on diets of algae and detritus in Fig. 6.9. Algal concentrations somewhere between 10 - 15 X 10^6 cells l^{-1} approximated SFG in the natural environment, whereas all detrital rations investigated resulted in an underestimation. Furthermore, the curves for laboratory data do not show the ultimate decline in production (SFG) with age as evident in field data.

Although food in the form of algae or detritus did not accurately predict field estimates of growth at the concentrations used, the range in laboratory SFG based on these diets did demonstrate an ability on the part of *D. serra* to exploit fully its growth potential in the short-term. Maximum growth and reproduction was realised at a ration of 30 X 10^6 *T. suecica* cells l^{-1} (6.06 mg organic DW l^{-1}), whilst short-term tolerance of severe starvation was indicated at a ration of 5 X 10^6 algal cells l^{-1} (1.01 mg organic DW l^{-1}).

P:B ratios given in Table 6.8 demonstrate very high rates of annual production in juvenile bivalves (0.01 g). In the field these animals increased their initial biomass 37 times. A similar turnover was estimated in the laboratory at an algal ration of 10 X 10^6 cells l^{-1}.

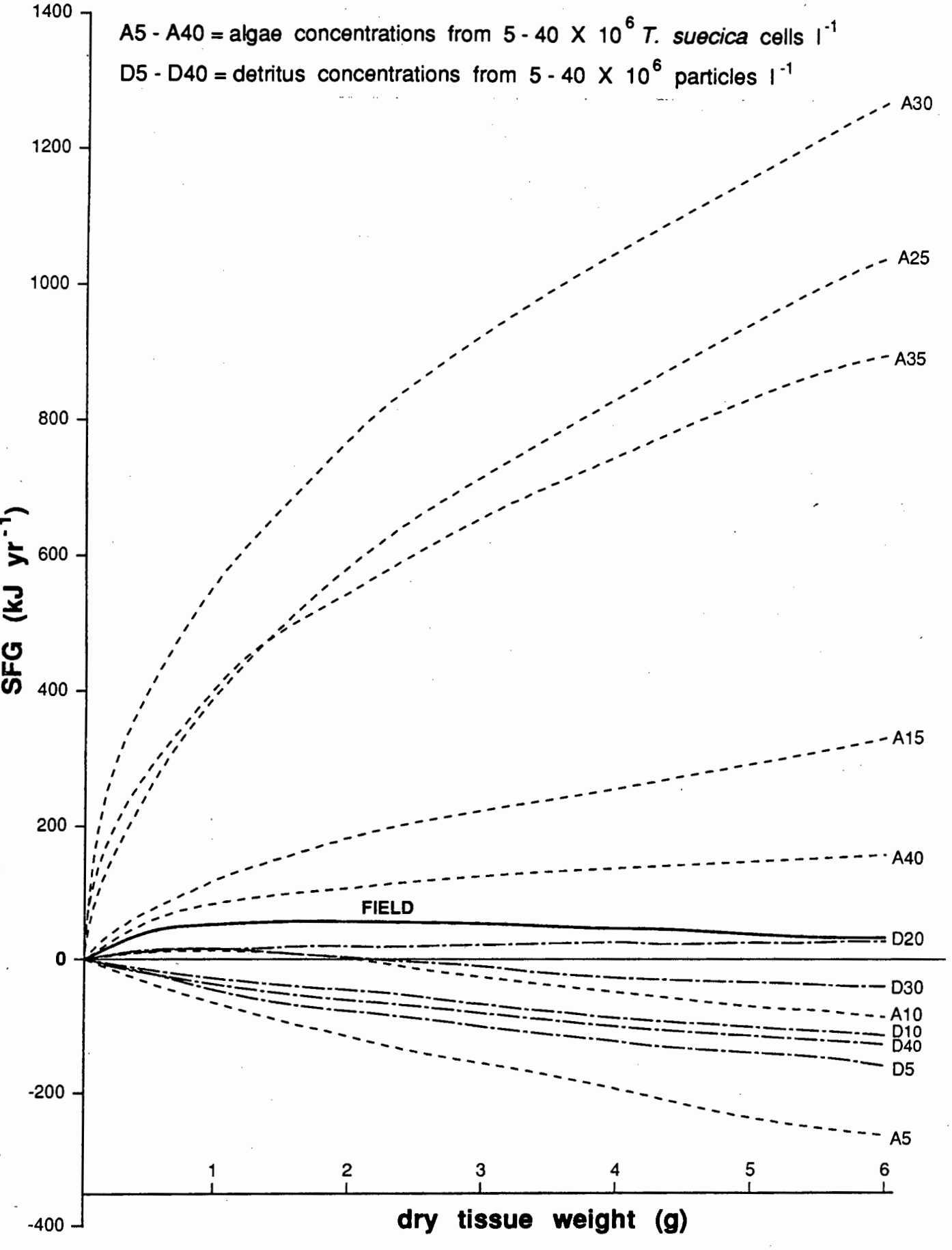

Fig. 6.9. Comparison between laboratory and field estimates of SFG (kJ yr^{-1}) in *D. serra* at Ouskip.

Table 6.8. P:B ratios from laboratory estimates of tissue production (P; kJ yr^{-1}) using algae and detritus as food. B = initial biomass of tissue (J). Field data from Table 6.7 are included here for comparison. " -- " refers to negative ratios.

DRY TISSUE WT (G)	ALGAL CONCENTRATION X 10^6 l^{-1}						
	5	10	15	25	30	35	40
0.01	--	32.14	81.04	194.28	417.86	281.45	77.34
0.05	--	10.96	35.57	92.46	172.03	117.74	29.39
0.10	--	6.51	25.09	67.59	117.35	80.65	18.64
0.50	--	1.17	11.14	32.39	38.94	34.32	7.67
1.00	--	0.15	7.73	23.47	33.39	23.59	5.09
2.00	--	--	5.42	16.95	22.62	16.07	3.21
3.00	--	--	4.26	14.01	18.08	12.91	2.56
4.00	--	--	3.81	12.32	15.45	11.05	2.12
5.00	--	--	3.45	10.92	13.49	9.64	1.75
6.00	--	--	3.09	10.22	12.36	8.88	1.66

DRY TISSUE WT (G)	DETRITUS CONCENTRATION X 10^6 l^{-1}					FIELD RATIOS
	5	10	20	30	40	
0.01	--	--	3.29	12.14	--	36.73
0.05	--	--	1.83	3.70	--	16.28
0.10	--	--	1.41	1.98	--	11.29
0.50	--	--	0.81	0.14	--	4.74
1.00	--	--	0.63	--	--	2.97
2.00	--	--	0.49	--	--	1.77
3.00	--	--	0.42	--	--	1.13
4.00	--	--	0.38	--	--	0.76
5.00	--	--	0.35	--	--	0.52
6.00	--	--	0.33	--	--	0.27

Both laboratory and field data clearly show that P:B ratios decline rapidly with increasing size. For example, adult animals beyond peak gonad-production demonstrated field P:B ratios of <1. The extremely high turnover ratios at the more concentrated algal rations cannot be expected to be maintained in the field on a long-term basis. These values only have meaning during short-term periods of high energy gain.

DISCUSSION

SHORT-TERM ESTIMATES OF SFG

This study has clearly demonstrated that dietary quality and quantity profoundly influence short-term laboratory estimates of SFG in *D. serra*. At comparable ration levels, whether expressed as mg total or organic DW l^{-1}, SFG on an algal diet surpassed that when feeding on less nutritious and more refractory seafoam detritus. This effect was most dramatic at optimum SFG, which was 50 times greater when algae were used as food. The principle cause of this contrast was the markedly depressed ingestion rates and, to a lesser extent, lower absorption efficiencies in detritus-fed animals (Chapter 4). At the same time, metabolic costs (R + U) were not greatly influenced by diet (Chapter 5), resulting in a small optimum energy gain in the balance of Ab - (R + U) when assimilating detritus. Moreover, this

small gain only occurred over a very restricted range of detrital concentrations, 5 - 8 mg DW l^{-1}. In contrast, SFG with algae was positive at all rations above 2 - 3 mg DW l^{-1}.

Although there have been a number of studies on the effects of food quality on isolated components of energy budgets, especially ingestion and absorption rates (Haven & Morales-Alamo, 1966; Winter, 1970, 1976; Foster-Smith, 1975; Bricelj & Malouf, 1984; Robinson *et al.*, 1984), few have quantified effects on SFG. In one such study, on the mussel *Aulacomya ater*, which inhabits kelp beds in close proximity to Ouskip beach (Stuart, 1982), SFG on a cultured-algal diet was compared with that when fed natural particulates, in this case synthesised from kelp fronds. The effect of each of these diets on growth in *A. ater* differed markedly from that observed in *D. serra*. Ingestion rates in *A. ater* were only slightly depressed when fed detritus. Of more significance however, was the maintenance of favourable absorption efficiencies at high detrital concentrations, whereas at high algal rations, efficiencies declined to zero. This subsequently resulted in similar rates of growth between *A.ater* fed kelp detritus and those provided with algae at low rations (<2 mg DW l^{-1}). At higher rations (2 - 6 mg DW l^{-1}), SFG declined with algae as food, but continued to increase with detritus.

These differences in laboratory SFG probably reflect species-specific responses to site-specific differences in

quality and composition of natural particulates. Kelp detritus is slightly inferior in quality to seafoam particulates in terms of percentage organics, energy content and C:N ratios (see Stuart, 1982). In terms of availability, kelp detritus is of equal or even greater importance as a food resource than is phytoplankton in the kelp beds inhabited by *A. ater* (Stuart *et al.*, 1982a, b; Newell & Field, 1983; Seiderer & Newell, 1985). The favourable SFG attained by *A. ater* when fed detritus, indicates that this species is able to efficiently exploit this impoverished but abundant food resource.

In the surf zone at Ouskip, phytoplankton, phytoplankton-derived detritus and seafoam are the major food materials available to *D. serra* (Chapter 4). This animal has the ability to exploit its natural resource opportunistically, by markedly increasing ingestion and growth potential in association with sporadic phytoplankton blooms. By contrast, *A. ater* appears to maintain a lower but longer-term and steadier positive growth over a wide concentration range of both phytoplankton and detritus.

Other studies concerned with the effect of food quality on SFG have used diets of cultured algae mixed with variable amounts of sedimentary silt from the natural environment. Growth in *M. edulis* and the clam *Spisula subtruncata* was generally higher on an algae + silt diet than one of pure algae (Mohlenberg & Kiorboe, 1981; Kiorboe *et al.*, 1981; Bayne *et al.*, 1987). In these studies it was found that the

potential reduction of food value by silt was counteracted by increasing the rate of some or all of the processes of clearance, ingestion and absorption. Furthermore, efficient pre-ingestive particle selection, a behaviour unlikely in *D. serra* (see Chapter 4), ensured that the most nutritious material was ultimately assimilated.

It would appear, therefore, that *D. serra* shows an enhanced ability over other species mentioned to exploit its environmental resources in the short-term when food value as well as quantity are optimal. This would enable rapid build-up of body reserves for later utilisation when food quality of surf particulates are insufficient to maintain growth and reproduction. This feeding strategy would compensate for the lack of pre-ingestive selection, a behaviour which enables some bivalves to obtain the most value from a poor diet without drastically increasing ingestion and/or absorption rates. The capacity to escalate consumption markedly would also be of significant advantage at times when reserves are diminished and there is an increase in the instantaneous maintenance requirements.

Of great relevance to understanding energy balance in *D. serra*, is a comparison between maintenance and optimum rations estimated in the laboratory and particulate concentrations found in Ouskip surf (see Table 4.1). In this instance, it is convenient to use Joules l^{-1} as a common measure of quality and quantity. Over a period of 15 months, surf particulates ranged from 7 to 107 J l^{-1}, the

latter value being obtained during a phytoplankton bloom in April, 1985. In the laboratory, the ration at which optimum SFG occurred on a algal diet was 155 J l^{-1}. With detritus as food, the best attainable SFG was realised at 54 J l^{-1}.

Since the optimal algal ration exceeded energy levels in the surf zone at Ouskip, it is doubtful whether the peak SFG measured on a diet of *T. suecica* would be attainable by *D. serra* in nature. By contrast, the optimum detrital ration corresponds to natural energy levels. Maximum SFG determined on this laboratory diet could thus be realised at Ouskip. Energy levels in the surf were closer however, to laboratory maintenance rations which were between 50 and 54 J l^{-1} on both diets. This implies that field SFG would only be marginally positive most of the time.

Relative partitioning of ingested energy between different physiological processes (Figs. 6.1 & 6.2) clearly showed that all sizes of *D. serra* released substantial quantities of energy in the form of faeces, especially at the moderate to high food concentrations investigated. When one considers the density of the *Donax* population at Ouskip (up to 986 g DW m^{-2}, Cook & Birkett, 1984), energy in egested material could contribute enormously towards total surf-particulate energy. Resuspended faeces may therefore represent a potential food resource for secondary processing by *D. serra*, especially after enrichment by colonising micro-organisms (Stuart *et al.*, 1982a).

GROWTH EFFICIENCIES

The efficiency with which optimum rations were converted into body energy was dependent on the size of *D. serra* and was strongly influenced by the quality of diet. When feeding on algae, small bivalves (0.1 g) displayed a growth efficiency of 70% and larger individuals (5 g), 50%. Ingestion of detritus resulted in much lower efficiencies of 8 and 3% respectively. Griffiths & Griffiths (1987), in summarising optimum K_1 values for a number of mytilid species, calculated a mean efficiency of 38% from a range of 0 to 57%. The corresponding mean optimum ration based mainly on algal diets was 1.1 mg DW l^{-1} (range = 0.3 - 4.5). Even taking into account the variation in animal sizes used by other workers, growth efficiencies in *D. serra* were above average on an algal diet. When feeding on detritus however, efficiencies were below average. Furthermore, optimum K_1-rations in *D. serra* (6 - 9 mg DW l^{-1}) were above the range given by Griffiths & Griffiths (1987) for other species.

In the light of previous discussion on opportunistic maximisation of energy gain when exposed to an enriched food source such as algae, it follows that the above-average growth efficiencies in *D. serra* could only be realised in the short-term, at the most for the 2 - 3 day duration of a phytoplankton bloom. In the long-term, efficiencies are probably closer to those established on the detrital diet.

It is to be expected that small *D. serra* were more efficient at converting food energy into body energy,

principally because of two physiological phenomena. Firstly, smaller organisms filter greater volumes of water per unit oxygen consumed than larger ones (Jorgensen, 1976b) and secondly, adults channel more surplus energy into gamete production than somatic growth (Bayne & Newell, 1983). When a wide size range of bivalves is tested, typical size-related differences in growth efficiencies range from about 35% in the adult of a species to approximately 50% in juveniles, a difference of some 15% (Thompson & Bayne, 1974; Widdows, 1978b; Navarro & Winter, 1982).

Although gross growth efficiencies in *D. serra* were above average, the percentage difference in relation to size was similar to published data (about 20%). However, this difference was proportionally diminished on a poorer diet, so that smaller *Donax* were assessed as being only 5% more efficient than adults. This is in sharp contrast to efficiencies in the kelp-bed mussel, *A. ater* in which K_1 values differed little between diets of algae and kelp detritus irrespective of size (Griffiths & King, 1979a; Stuart, 1982). On the other hand, growth efficiencies in *M. edulis* (calculated from Tables 4 & 5 in Bayne *et al.*, 1987) increased with the organic content of food, but only after the mussels had been exposed to experimental diets for 2 weeks.

FIELD ESTIMATES OF GROWTH AND REPRODUCTION

Although physiological budgeting based on laboratory experiments provides a convenient and relatively instantaneous measure of growth potential, it has three major drawbacks. Firstly, it is not possible to distinguish between somatic and reproductive growth, a distinction which is valuable in realising partitioning of surplus energy between juvenile, mature and aging individuals. Secondly, it is recognised that energy balance based on laboratory foods does not adequately mimic energy gain and loss in the field. Thirdly, short-term estimates of laboratory SFG are most often extrapolated into long-term estimates without regard for temporal (seasonal) variability in budget components brought about by both exogenous and endogenous factors such as temperature cycles and changes in food quality and availability (Worrall *et al.*,1983; Hawkins & Bayne, 1984; Hawkins *et al.*, 1983, 1985; MacDonald & Thompson, 1986; Chapters 4 & 5). All three disadvantages are clearly demonstrated in the present study.

The Gompertz growth equation for *D. serra* demonstrated two features not apparent from laboratory SFG estimates; asymptotic size is reached after 5 to 6 years and the growth rate declines with age, especially after the third year. Similar growth patterns have also been observed in other *Donax* populations along the west coast of South Africa (de Villiers, 1975b), as well as on the south-eastern shore

(Donn, 1986) and would therefore appear characteristic of *D. serra*.

The Ouskip population did, however, display a slightly higher growth rate than these other populations in terms of shell width at the end of each of 5 consecutive years (Table 6.9). Intraspecific differences in growth rates are not uncommon and are usually attributed to environmental factors, especially the nature and cycle of food resources (Griffiths & Griffiths, 1987). Unfortunately, lack of data prohibits a correlation between variation in growth rates and local food availability. Nevertheless, such a correlation has been clearly demonstrated for a number of species, including *Scrobicularia plana* (Worrall *et al.*, 1983), *Placopecten magellanicus* (MacDonald & Thompson, 1985), *Crassostrea gigas* (Brown, 1988) and *Macoma balthica* (Thompson & Nichols, 1988).

Table 6.9. Comparison of growth rates (mm yr^{-1}) between three separate populations of *D. serra* in terms of shell width (mm) after each of 5 consecutive years .

YEAR	OUSKIP (this study)	WEST COAST (de Villiers, 1975b)	SOUTH COAST (Donn, 1986)
1	17	14	15
2	32	28	28
3	44	38	38
4	52	43	43
5	55	45	46

The irregularity of spawning in *D. serra* is consistent with histological evidence of non-cyclic periods of prolonged gamete release (de Villiers, 1975b; Birkett & Cook, 1987). Indeed, a comparison of Fig. 6.7 with Fig. 6.10 (after Birkett & Cook, 1987), reveals a remarkably close correlation between periods of weight loss in a standard 45-mm bivalve and increased frequency in spawning, especially in 1982 and 1983. Similar association between histological preparations of gonad tissue and changes in overall somatic weight have been observed in *S. plana* (Worrall *et al.*, 1983), *M. edulis* (Hawkins *et al.*, 1985) and *P. magellanicus* (MacDonald & Thompson, 1986). However, for these species a close association was more likely since all exhibited predictable reproductive cycles.

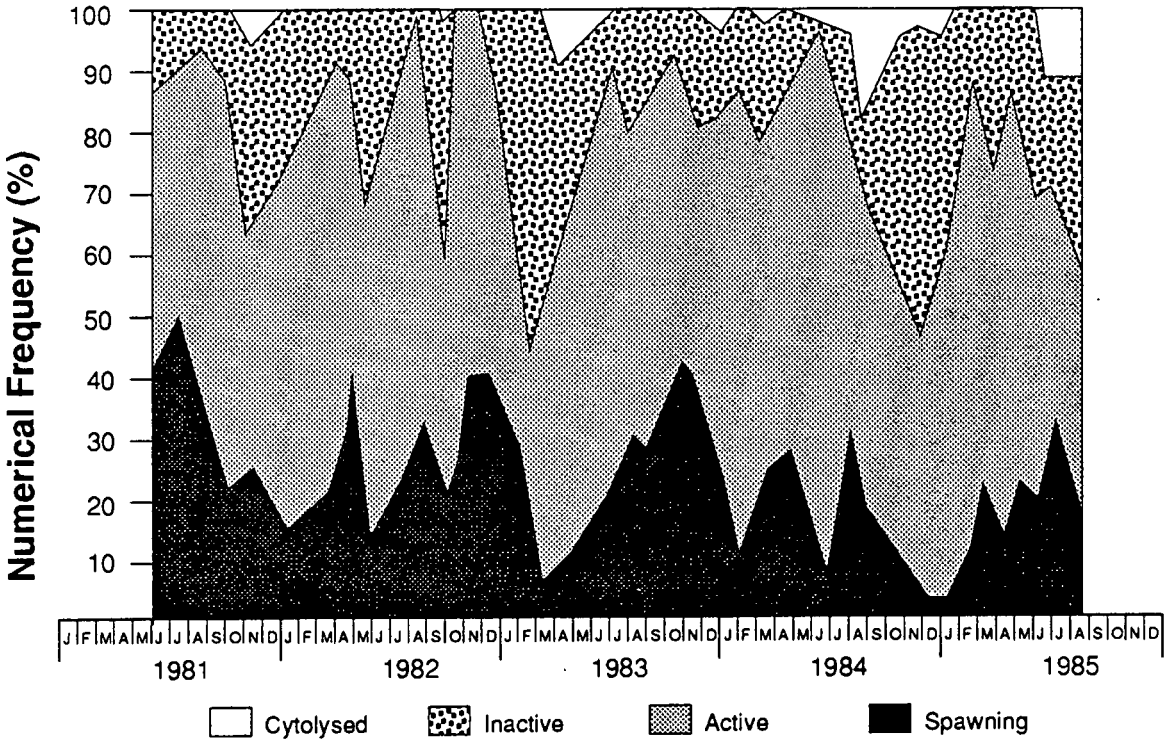

Fig. 6.10. Gametogenic cycle of *D. serra* (after Birkett & Cook, 1987).

Nonetheless, these findings clearly support the reliability of using monthly regressions of shell-dimension to weight to estimate P_r.

During 1982 and 1983 there was a temperature anomaly on the west coast of southern Africa in which mean-monthly inshore temperatures rose by 2 to 5°C (Maxwell & Rattey, 1983; Walker *et al.*, 1984). It has been suggested that this anomaly was responsible for an increase in the number of spawning and active *D. serra* (Birkett & Cook, 1987). Nevertheless, the reproductive output by individual *D. serra* were not observed to increase during these years relative to 1984/85. For instance, a 45-mm bivalve released 25 and 35 kJ of gametes in 1982 and 1983 respectively, compared with 50 and 35 kJ in 1984 and 1985. These data further emphasise that the amount spawned by any one size of *D. serra* is not consistent from year to year. This, coupled with changes in the number of active/spawning and inactive/cytolysed bivalves would determine the reproductive output of Ouskip individuals in response to environmental conditions.

Comparison between laboratory and field estimates of SFG in *D. serra* dramatically demonstrates the need for circumspection when extrapolating data. This is especially so when short-term data sets (i.e. J hr^{-1}) are multiplied by 24 and then 365 to obtain daily and annual estimates. It was quite clear that optimal SFG in kJ hr^{-1} on a cultured-algal diet grossly overestimated growth in the natural environment, whereas with detritus as food, field growth was

moderately underestimated (Fig. 6.9). Risk of extrapolation was even more vividly depicted in the unrealistic optimum annual turnover ratios (Table 6.8). These very high P:B ratios lend credence to the earlier suggestion that algal-SFG signified immediate rather than long-term optimisation of a food resource. In contrast, the assertion that detrital-SFG was more indicative of long-term optimisation makes extrapolation to annual estimates more justifiable when using this type of food.

Underestimation of natural growth in the laboratory based on detritus may have been less if *D. serra* had been exposed to the impoverished seafoam diet instead of a mixture of algae and detritus prior to measurement of ingestion and absorption. This is suggested in the light of the finding of Bayne *et al.* (1987) which clearly demonstrated dietary acclimation in *M. edulis* after 2 weeks exposure to low-organic food. After this time, ingestion rates and absorption efficiencies increased, whilst energy utilisation declined so that SFG compared favourable with that on a richer organic diet. Robertson *et al.* (1984) also recorded acclimation, although less convincingly, in *Spisula solidissima* held at high concentrations of suspended clay for 21 days.

Physiological compensation in *D. serra* may be indicated by the fact that field absorption efficiency was higher (80%) than the maxima recorded on a diet of either algae (74%) or foam detritus (57%) in the laboratory (see Chapter

4). Constant exposure to surf particulates could promote endogenous conditioning, so that energy yield may be improved to levels greater than the laboratory experiments indicated. Such physiological compensation may, partially, explain the discrepancy between estimated growth on a near-natural diet of detritus and field growth.

Studies attempting to evaluate the reliability of energy budgets by comparing estimated (i.e. SFG) and actual growth rates in bivalves, provide contradictory results. Some laboratory experiments using a variety of algal diets have failed to obtain the maximum growth rates observed in nature (Tenore *et al.*, 1973; Winter & Langdon, 1976; Mohlenberg & Kiorboe, 1981; Kiorboe *et al.*, 1981). Addition of natural silt to algae has, in some instances, resulted in growth rates more comparable to natural growth (Winter, 1976; Kiorboe *et al.*, 1980, 1981; Mohlenberg & Kiorboe, 1981). Yet in other studies, bivalves presented with algae exhibit SFG similar to actual growth rates (Riisgard & Randlov, 1981; Navarro & Winter, 1982; Hawkins *et al.*, 1985; Hummel, 1985). Further contrast is provided by the apparent gross overestimation of actual growth in *D. serra* fed algae and comparable underestimation when presented with foam detritus.

However, it is evident from the present study that the descriptive validity of SFG as an index of natural production is not simply a function of the quality of laboratory food. At certain concentrations of both algae

and detritus that are not necessarily optimal, SFG would be very similar to actual growth. For instance, a ration of algae between 2 and 3 mg organic DW l^{-1} (51 - 77 J l^{-1}) would provide an annual SFG close to field growth, whilst a detrital ration of about 3 mg organic DW l^{-1} (54 J l^{-1}) would have a similar result.

It is highly likely therefore, that much of the variability in SFG results in general may be attributed to a **combination** of the quantity and quality of food used. This should be taken into account when attempting to estimate actual field growth from energy budgets. In this context it may be significant that the laboratory rations at which SFG and real growth were similar in *D. serra* (2 -3 mg organic DW l^{-1}), approximate the mean particulate level at Ouskip (3.7 mg organic DW l^{-1}) [see Table 4.1]. Clearly, estimated growth in *D. serra* only approximates field growth over a restricted range of food quantity and quality. Outside this range, SFG would be interpreted as either overestimating or underestimating real growth. Careful attention to the type and concentration of food used in energy budgets studies would go a long way to providing reasonable predictions of natural growth.

CONCLUSIONS

1). A bivalve species' potential to optimise SFG in its own environment depends on specific physiological adaptations to the composition, nutritional content and palatability of the natural resources. In the surf at Ouskip, the food resource is predominantly phytoplankton-derived detritus and seafoam augmented by phytoplankton blooms. *D. serra* demonstrates an ability to exploit this resource by opportunistically increasing SFG in the short-term in response to sporadic phytoplankton blooms. Phytoplankton represents a nutritious food source and as such would significantly supplement an otherwise impoverished diet of surf-zone detritus.

2). The extent of short-term optimisation of SFG by *D. serra* was reflected in growth efficiencies higher than previously recorded among bivalve species. In the longer term however, lower growth efficiencies can be expected in association with a predominantly detrital diet.

3). The validity of using monthly regressions of shell width to tissue weight to identify periods of weight loss synonymous with spawning events, was substantiated by a close correlation between the timing of such events and the periods of gamete release discernable from histological preparations. This correlation was especially remarkable since the timing of gamete release in *D. serra* is prolonged and non-cyclic, and hence unpredictable.

4). Comparison between laboratory and field estimates of SFG, growth efficiencies and P:B ratios in *D. serra*

dramatically demonstrates the need for circumspection when extrapolating data. Optimal production on a cultured algal diet overestimated growth in the natural environment whereas with detritus as food, field growth was moderately underestimated. The validity of SFG as an index of natural production is therefore a function of the **combination** of food quality and quantity.

CHAPTER SEVEN

EFFECTS OF TEMPERATURE AND CHLORINE ON FEEDING, METABOLIC EXPENDITURE AND SCOPE FOR GROWTH AND REPRODUCTION

INTRODUCTION

Within the range of normal environmental temperatures, many marine molluscs are able to adjust components of energy gain and loss, such that scope for growth and reproduction is optimised (Widdows & Bayne, 1971; Bayne *et al.*, 1976; Newell *et al.*, 1977; Newell, 1980; Buxton *et al.*, 1981; Thompson & Newell, 1985). Some achieve this by enhancing clearance rates to compensate for an increase in metabolic energy costs as environmental temperatures rise seasonally or along a latitudinal gradient (Newell & Kofoed, 1977a, b; Newell *et al.*, 1977). Others may show a compensatory increase in both absorption efficiency and clearance rate to offset increased energy expenditure at high temperatures (Winter, 1969; Newell, 1979, 1980; Newell & Branch, 1980). There are also species that display a uniform rate of clearance over the normal range of thermal tolerance, despite the fact that their metabolic rate increases with temperature (Griffiths, 1980a, 1981a). Clearly, therefore, changes in temperature result in species-specific responses in both the rate of energy acquisition through feeding and assimilation and of energy expenditure through respiration and excretion. The relative magnitude of these changes will determine whether there is a net improvement or decline in SFG at different temperatures.

The present study was designed to determine the manner of and extent to which *D. serra* is able to adjust components of its energy budget when exposed to temperatures and

chlorine levels characteristic of the power station discharge plume. The plume presents an environment with steep thermal gradients together with a less well-defined dispersion of chlorine. Chlorine dissipates quickly in sea water and consequently some areas of the plume confer only thermal stress while others, especially near the discharge point, pose an influence of both high temperature and sub-lethal levels of chlorine (see Chapter 1).

Experiments were designed so that rates of clearance, ingestion, absorption, respiration and excretion measured at an ambient temperature of 15°C (Chapters 4, 5, & 6) served as controls against which rates at 20 and 25°C, with or without chlorine, could be compared. Subsequent estimates of SFG were taken as indicators of the integrated response of physiological activities to thermal stress and chlorine contamination.

MATERIAL AND METHODS

MAINTENANCE AND PREPARATION OF ANIMALS

D. serra, collected from Ouskip beach between September and December 1986, were kept at 15°C in flow-through aquaria with sand and provided with a mixed diet of algae and detritus. After one week of laboratory confinement, animals were divided into 6 groups, each containing a representative size range of individuals. Groups were then exposed to either 15, 20 or 25°C, with or without chlorine, for two

weeks to allow for possible acclimation prior to experimentation.

Exposure to high temperatures was gradually attained by an increase of 2°C per day whilst chlorine, in the sublethal range of 0.1 - 0.3 ppm, was administered continuously as described in Chapter 2. During the two-week period, *Tetraselmis suecica* was provided as food on a daily basis. The water was replaced every second day with fresh sea water at the appropriate temperature and chlorine concentration. Animals were always used within the first week after the 2-week acclimation period.

T. suecica was provided at a concentration of 20 - 25 X 10^6 cells l^{-1} (= 4.5 - 6.5 mg DW l^{-1}). This ration was chosen since it represents the quantity and quality of food at which optimal scope for growth and reproduction (SFG) were measured in the laboratory (see Chapter 6). It is assumed that at sub-optimal rations, effects of temperature and chlorine on feeding, metabolic expenditure and energy balance are likely to be more severe.

FOOD ASSIMILATION

Rates of clearance (CR), ingestion (IR) and absorption (AR) were measured in the same manner as described in Chapter 4. Clearance rates were quite variable during the 8-hr experiments. As described in Chapter 4, final rates were calculated as means for 20-min periods over 8 hours to compensate for this variability. The ash-ratio method

(Conover, 1966) was again used to determine absorption efficiencies (AE).

Experiments at 15°C served as controls against which exposure to chlorine at this temperature and exposure to 20 and 25°C with or without chlorine was evaluated. All experiments were conducted in a constant-temperature room.

METABOLIC EXPENDITURE

Rates of aerobic respiration (RR) and ammonia-N excretion (U) were measured as in Chapter 5. Rates of faecal production (F) were determined from the difference between ingestion and absorption rates.

Three levels of metabolic activity were again recognised during measurements of oxygen consumption; standard (individuals that had been starved for 2 weeks prior to experiments), and two routine levels, namely the fed condition (digesting/assimilating food) and feeding (clearing and ingesting particles as well as assimilation). Unfortunately at 15°C with chlorine, standard metabolic rate could not be assessed since *D. serra* did not maintain open siphons and active pumping for long enough to allow for reliable measurements of oxygen consumption. Rates of ammonia excretion were only measured in animals that had recently fed on algae.

As with the feeding experiments, temperature was controlled by housing the flow-through respirometer (see Fig. 5.1) in a constant-temperature room. Where applicable,

chlorine was introduced into the bottom reservoir. Excretion experiments were conducted in glass beakers in the same room.

RATE-TEMPERATURE CURVES

Since aerobic respiration was shown to be the major source of energy loss to *D. serra* (Chapter 6), investigation into the effects of acute temperature change (T_e) on oxygen consumption was relevant. Adult *D. serra* (>35 mm shell width), acclimated to 15°C for 2 weeks, were directly exposed to 10, 20, 25 and 30°C and oxygen uptake monitored. This procedure was followed for individuals in the starved, fed and feeding condition. By contrasting these short-term respiratory responses to those which followed the 2-week acclimatisation period, the extent of possible acclimation could be ascertained.

A second set of experiments was designed to investigate the nature of potential thermal acclimation, namely whether acute RT curves translated laterally or rotated with an increase in exposure temperature (see Bayne, 1976; Bayne & Newell, 1983). Adult bivalves were exposed for 2 weeks to 10, 15, 20, 25 and 30°C (referred to as T_a), during which time *Tetraselmis suecica* was provided as food. In the case of the high temperatures (20, 25 and 30°C), exposure was gradually reached by a 1-2°C increase per day over the 2-week period. Oxygen consumption of fed individuals from each T_a was determined on acute exposure to a temperature

range (T_e) following the sequence 20, 10, 25, 15 and 30°C. This minimised the possibility of short-term acclimation effects during experiments.

At any exposure temperature, a bivalve was allowed to settle for one hour in the respiration chamber, through which sea water flowed at the appropriate temperature. Three consecutive determinations of oxygen consumption were made, whereafter temperature was changed to the next in sequence. At all times a chamber without an animal served as a control. After completion of experiments, body tissue was dried at 60°C for 2 days so that all data could be expressed as ml O_2 per gram dry weight.

SCOPE FOR GROWTH AND REPRODUCTION

Energy available for growth and reproduction (SFG) at 15, 20 and 25°C, with or without chlorine, was determined by balancing rates of energy acquisition (Ab = IR X AE) against expenditure (RR + U)). Energy lost in egested faeces (F) was determined from the difference between energy ingested and that absorbed.

As described in Chapter 6, calculation of energy balance was based on the equation of Winberg (1956), namely SFG = Ab - (RR + U). Conversion to energy values was obtained using ratios given in Chapter 4 for the dry weight of *Tetraselmis suecica* and in Chapter 5 for ml O_2 consumed and ug NH_4-N excreted. SFG data were also used to assess the effect of temperature and chlorine on growth

efficiencies (Calow, 1977). The two efficiencies calculated were gross-growth efficiency, (K_1) and net-growth efficiency, (K_2) (see Chapter 6).

RESULTS

FOOD ASSIMILATION

Clearance and ingestion rates

Rates of clearance (CR, l hr^{-1}), ingestion (IR) and absorption (AR) [mg DW l^{-1}] are given in Table 7.1 in the form of allometric equations relating to dry tissue weight at 15, 20 and 25°C, with or without chlorine. The highly significant ($P > 0.005$) Pearson product-moment correlation coefficients (r) demonstrate the strength of the positive relationship between body size and feeding rates. Equations for absorption rates, although presented in Table 7.1, will be examined later.

To test whether temperature increase and chlorine had a significant effect on CR and IR, allometric regressions were subjected to covariance analysis and a Newman-Keuls multiple range test (Tables 7.2 & 7.3 respectively). These analyses focused on three aspects of the data: (1) effect of elevated temperature without chlorine or (2) with chlorine, and (3) the effect of chlorine as a single stress factor at either 15, 20 or 25°C.

Table 7.1. The *a*- and *b*-values of allometric regressions (RATE = aW^b, W = dry body weight, g) expressing the effect of temperature and chlorine on FOOD ASSIMILATION as given by the rates of clearance (CR, l hr^{-1}), ingestion (IR) and absorption (AR) in mg dry algae hr^{-1} at a concentration of 20 - 25 X 10^6 cells l^{-1}.

	NO CHLORINE				WITH CHLORINE			
	a	*b*	n	r	*a*	*b*	n	r
CR								
15°C	1.050	0.550	22	0.93	0.077	0.741	32	0.92
20°C	1.080	0.740	22	0.87	0.227	0.450	17	0.83
25°C	1.188	0.760	34	0.92	0.531	0.490	19	0.90
IR								
15°C	4.551	0.570	22	0.94	0.375	0.686	32	0.90
20°C	4.539	0.750	20	0.93	1.075	0.470	17	0.88
25°C	5.191	0.710	31	0.93	2.442	0.453	19	0.89
AR								
15°C	2.960	0.570	22	0.94	0.270	0.686	32	0.90
20°C	1.590	0.750	20	0.93	0.591	0.470	17	0.88
25°C	1.130	0.710	31	0.93	0.659	0.453	19	0.89

In non-chlorinated experiments, there was no significant difference between slopes within each ANOCOVA thus allowing computation of mean weight coefficients (b_c) of 0.66 for clearance and 0.65 for ingestion. Furthermore, elevations (*a*-values) were similar, signifying that both rates of CR and IR were temperature independent. The 2-week thermal regime prior to experiments was obviously sufficient to allow acclimation.

In the presence of chlorine however, ANOCOVA identified some highly significant differences in CR and IR that were temperature-related. Considering firstly analysis of clearance rates, an increase in temperature significantly affected the weight coefficient, *b* (Table 7.2). The subsequent Newman-Keuls (NK) procedure however, failed to detect differences between any pair of *b*-values. According to Zar (1982), the outcome from the more powerful ANOCOVA should take preference over multiple range testing. Nonetheless, it is obvious that among CR slopes, that at 15°C (0.74) was notably greater than that at 20°C (0.45) or 25°C (0.49).

Although differences between elevations could not be tested statistically (Table 7.2), weight-specific clearance rates appeared more sensitive to temperature in a chlorinated environment. There was a two- to three-fold temperature-related increase in CR compared with only a fractional increase without chlorine.

Table 7.2. Effect of temperature and chlorine on CLEARANCE RATES (CR, 1 hr^{-1}): analysis of the significant difference between the slopes (*b*-values) and elevations (*a*-values) of allometric equations for CR at 15, 20 and 25°C with or without chlorine (see Table 7.1 for equations). Analysis procedure follows Zar (1982) using $\log_{10}$ transformed data.

ANALYSIS OF COVARIANCE $P < 0.01$

	NO CHLORINE Between 15, 20 & 25°C					WITH CHLORINE Between 15, 20 & 25°C			
Between	k	DF	F_s	F	b_c	k	DF	F_s	F
b-values	3	72	4.31	4.92	0.66	3	62	6.13	4.98
a-values	3	72	0.32	4.92					

NEWMAN-KEULS MULTIPLE RANGE TEST
Determination of which slopes for regressions with chlorine are significantly different from one another

Between pairs (°C)	q	p	$q_{0.01,62}$
15 & 20	4.10	3	4.28
15 & 25	3.38	2	3.76
20 & 25	0.63	2	3.76

STUDENT-*t* TEST
Comparing CR with or without chlorine at the same temperature

Temperature (°C)	Between slopes (*b*)				Betw elevations (*a*)		
	t_s	$t_{0.01(2)}$	DF	b_c	t_s	$t_{0.01(2)}$	DF
15	-2.52	±2.66	50	0.62	23.34	±2.66	51
20	2.50	±2.73	35	0.58	11.66	±2.73	36
25	3.13	±2.66	49				

Differences in slope and elevation indicate that in response to high temperatures with chlorine, the increase in clearance rates is greater in small *D. serra* relative to large ones. At 15°C, a 3 g bivalve filters at 12.5 times the rate of a 0.1 g individual, whereas at 25°C this size-related difference is much reduced, the rate of the larger animal being only 5.2 times greater than that of the 0.1 g one.

Considering the effect of chlorine alone on CR, the Student-*t* procedure accepted homogeneity between matched slopes at 15°C and at 20°C (Table 7.2). Subsequent analysis of elevations demonstrated that at these temperatures, chlorine significantly depressed clearance rates, to a greater extent at 15°C (93% reduction) than at 20°C (79%). At 25°C, chlorine significantly reduced the slope and although this prevented analysis of differences in elevation, weight-specific rates were also much lower (only 0.46 times that without chlorine). The overall effect of chlorine was thus a reduction of CR at all temperatures but at 25°C, this was more pronounced in large than in small *D. serra*.

As far as the ingestion of algae was concerned, the size-rate relationship was not altered by a combination of temperature elevation and chlorine, a common *b*-value of 0.54 being computed between temperatures (Table 7.3). At the same time, however, weight-specific IR (mg hr^{-1} g^{-1}), like CR, increased in response to an increase in temperature.

Table 7.3. Effect of temperature and chlorine on INGESTION RATES (IR, mg dry algae hr^{-1}): analysis of the significant difference between the slopes (*b*-values) and elevations (a-values) of allometric equations for IR at 15, 20 and 25°C with or without chlorine (see Table 7.1 for equations). Analysis procedure follows Zar (1982) using $\log_{10}$ transformed data.

ANALYSIS OF COVARIANCE $P < 0.01$

	NO CHLORINE Between 15, 20 & 25°C					WITH CHLORINE Between 15, 20 & 25°C				
Between	k	DF	F_s	F	b_c	k	DF	F_s	F	b_c
b-values	3	67	2.94	4.95	0.65	3	62	4.96	4.98	0.54
a-values	3	69	0.56	4.92		3	64	142.56	4.98	

NEWMAN-KEULS MULTIPLE RANGE TEST
(significant difference between a-values with chlorine)

Between pairs (°C)	q	p	$q_{0.01,62}$
15 & 20	8.85	3	4.28
15 & 25	5.51	2	3.76
20 & 25	3.34	2	3.76

Conclusion 20 = 25

STUDENT-*t* TEST
Comparing IR with or without chlorine at the same temperature

Temperature (°C)	Between slopes (*b*)				Betw elevations (a)		
	t_s	$t_{0.01(2)}$	DF	b_c	t_s	$t_{0.01(2)}$	DF
15	-1.54	+2.66	50	0.64	15.87	+2.66	51
20	2.84	+2.75	33				
25	3.33	+2.66	46				

NK tests revealed that when exposed to chlorine, ingestion at 15°C for a 1-g animal (0.38 mg hr^{-1}) was significantly lower than at either 20°C or 25°C. Differences between rates at these two high temperatures (1.08 & 2.44 mg hr^{-1} respectively) were however, insignificant.

At 15°C, chlorine itself had no marked effect on the size-IR correlation, but it did influence *b*-values at 20°C and 25°C. At these temperatures coefficients were much lower with chlorine (0.47 & 0.45, respectively), than without (0.75 & 0.71) [Table 7.3]. As may be expected from CR data, IR per unit weight at any one temperature was greatly depressed by chlorine, especially in large bivalves, although this effect could only be statistically substantiated at 15°C.

Absorption efficiency and rates

Absorption efficiencies (AE) of algae are given in Table 7.4 together with statistical analysis of differences relating to temperature and chlorine. There was a highly significant decline in mean efficiencies with increase in temperature of sea water free of chlorine. Efficiencies declined from 65% at 15°C to only 22% at 25°C, whilst with chlorine present, the corresponding decline was from 72% to 27%.

During experiments it was observed that with temperature elevation, the time period between the ingestion of algae and the appearance of algal-derived faeces was markedly shortened. At the same time the proportion of intact algal cells in faeces increased, so that the

intestinal fraction was more obvious than the glandular part. Intestinal faeces refers to material that has only passed through the intestine (reduced digestion and absorption) whilst the glandular part is that which has been retained in the digestive gland prior to egestion (greater digestion and absorption) [Calow, 1975; Van Weel, 1961; Widdows *et al*, 1979; see Chapter 4).

At any one temperature efficiencies were lower without, than with chlorine, although this could only be statistically validated at 20°C. Higher AE with chlorine may be partially attributed to two features of faecal production. Firstly, with chlorine, faeces took a longer time to appear following algal ingestion (usually double the time recorded at the same temperature without chlorine); this suggests a longer gut-residence time with the likelihood of more efficient absorption. Secondly, faeces were very fragmented and disintegrated rapidly on release into chlorinated sea water; this could indicate poor mucus-binding, a condition likely to reduce the organic content of faeces and thereby, according to the Conover ratio (Conover, 1966), increase the estimation of AE. Furthermore, the production rate of faeces was far more inconsistent among individuals when chlorine was present. This may account for the greater standard deviations around averaged efficiencies (Table 7.4).

Table 7.4. Absorption efficiencies (%) and statistical analysis of the effect of temperature (15 - 25°C) and chlorine (0.1 - 0.3 ppm) when feeding on *T. suecica* at a concentration of 20 - 25 X 10^6 cells l^{-1}. SD = one standard deviation either side of the mean; n = number of samples.

PERCENTAGE ABSORPTION EFFICIENCY

TEMPERATURE	NO CHLORINE			WITH CHLORINE		
°C	%	SD	n	%	SD	n
15	65.02	6.69	6	72.13	11.25	10
20	35.15	9.69	10	55.01	16.77	9
25	21.85	9.31	11	27.02	11.94	12

STATISTICAL ANALYSIS (P <0.05)

ANOVA 1

NO CHLORINE Between 15, 20 & 25°C				WITH CHLORINE Between 15, 20 & 25°C			
k	DF	F_s	F	k	DF	F_s	F
3	24	44.69	3.40	3	28	32.47	3.34

NEWMAN-KEULS TEST

Between	q	p	$q_{0.05,24}$	Between	q	p	$q_{0.05,28}$
15 & 20	9.12	2	2.92	15 & 20	3.98	2	2.89
15 & 25	13.35	3	3.53	15 & 25	11.22	3	3.49
20 & 25	4.72	2	2.92	20 & 25	6.75	2	2.89

STUDENT-t TEST

(Between non-chlorine & chlorine treatments, same temp)

Temperature (°C)	t_s	$t_{0.05\ (2)}$	DF	Sign. diff.
15	±1.38	±2.15	14	No
20	±3.21	±2.11	17	Yes
25	±1.11	±2.08	21	No

As a consequence of a temperature-related reduction in efficiencies, absorption rates also declined between 15 and 25°C, even though ingestion rates increased (Table 7.1). With chlorine present, AR increased slightly with higher temperature, simply because the corresponding decline in absorption efficiencies was of insufficient magnitude to reverse the highly significant positive correlation between ingestion rate and temperature. At any one temperature, AR was much lower in chlorinated water, but this resulted not from depressed AE but rather from reduced clearance and ingestion rates.

The statistical analysis of effects of temperature and chlorine on AR is summarised in Table 7.5. The size-rate relationship was not significantly changed by temperature elevation even with chlorine. The negative correlation between AR and temperature was significant with each 5°C difference. With chlorine present, the increase that occurred in absorption rates was only significant between 15°C and the two higher temperatures. Student-*t* test showed that at 15°C the chlorine-related suppression of AR was highly significant while at 20 and 25°C, rates declined to a greater extent in large than in small *D. serra*.

Table 7.5. Effect of temperature and chlorine on ABSORPTION RATES (AR, mg dry algae hr^{-1}): analysis of the significant difference between slopes (*b*-values) and elevations (*a*-values) of allometric equations for AR at 15, 20 and 25°C with or without chlorine (see Table 7.1 for equations). Analysis procedure follows Zar (1982) using $\log_{10}$ transformed data.

ANALYSIS OF COVARIANCE $P < 0.01$

	NO CHLORINE Between 15, 20 & 25°C					WITH CHLORINE Between 15, 20 & 25°C				
Between	k	DF	Fs	F	b_c	k	DF	Fs	F	b_c
b-values	3	67	2.93	4.95	0.63	3	62	4.96	4.98	0.54
a-values	3	69	39.75	4.92		3	64	41.37	4.98	

NEWMAN-KEULS MULTIPLE RANGE TEST
(significant difference between a-values)

Between pairs (°C)	NO CHLORINE			WITH CHLORINE		
	q	p	$q_{0.01,67}$	q	p	$q_{0.01,62}$
15 & 20	6.86	2	3.76	9.76	3	4.28
15 & 25	12.53	3	4.28	11.59	2	3.76
20 & 25	4.20	2	3.76	1.72	2	3.76

STUDENT-*t* TEST
Comparing AR at the same temperature with and without chlorine

Temperature (°C)	Between slopes (*b*)				Betw elevations (a)		
	t_s	$t_{0.01(2)}$	DF	b_c	t_s	$t_{0.01(2)}$	DF
15	-1.55	+2.66	50	0.61	22.22	+2.66	51
20	2.83	+2.75	33				
25	3.30	+2.70	46				

METABOLIC EXPENDITURE

Aerobic respiration rates (RR) of starved, fed and feeding *D. serra*, as well as ammonia excretion rates (U) of recently-fed animals, are given in Table 7.6. For all nutritional states and temperatures (with and without chlorine) there was a positive correlation between size and rate, which proved significant according to the Pearson Product-moment correlation coefficient ($P<0.005$). The same statistical procedure as used on feeding data (Tables 7.2, 7.3 & 7.5) was applied in analysing the effect of body size, temperature and chlorination on RR (Tables 7.7 & 7.8) and U (Table 7.9).

Oxygen consumption

At all three activity levels in non-chlorinated experiments, weight-specific O_2 uptake rates increased between 15 and 25°C (Table 7.6). At any one temperature, rates were lowest in starved individuals and maximal in those feeding, the difference reflecting, in part at least, the mechanical and physiological costs of processing algae.

With addition of chlorine, respiration rates could not be measured reliably in starved animals, since pumping activity and hence water transport were extremely erratic. When *D. serra* was in a better physiological state after being fed or while feeding, chlorine did not retard pumping activity in the same manner and O_2 consumption could be monitored. Rates were clearly depressed by chlorine, but even so, routine metabolism was still temperature-dependent.

Table 7.6. The *a*- and *b*-values of allometric regressions (RATE = $a \cdot W^b$, W = dry body weight, g) expressing the effect of temperature and chlorine on METABOLIC EXPENDITURE as given by rates of respiration (RR, ml O_2 hr^{-1}) when starved, fed and during feeding and ammonia-N excretion (U, ug hr^{-1}) when recently fed. Animals were supplied with a concentration of 20 - 25 X 10^6 *T. suecica* cells l^{-1}.

	NO CHLORINE				WITH CHLORINE			
	a	*b*	n	r	*a*	*b*	n	r
RR (starved)								
15°C	0.27	0.69	44	0.99				
20°C	0.31	0.70	44	0.98				
25°C	0.36	0.71	44	0.99				
RR (fed)								
15°C	0.37	0.70	44	0.98	0.24	0.65	44	0.97
20°C	0.45	0.70	44	0.98	0.27	0.73	44	0.98
25°C	0.46	0.72	44	0.98	0.38	0.71	44	0.98
RR (feeding)								
15°C	0.45	0.76	44	0.98	0.30	0.66	44	0.97
20°C	0.46	0.71	44	0.98	0.33	0.78	44	0.99
25°C	0.59	0.73	44	0.99	0.38	0.76	44	0.98
U (fed)								
15°C	39.14	0.55	13	0.89	5.47	0.47	13	0.97
20°C	52.26	0.49	13	0.95	39.87	0.46	13	0.99
25°C	66.44	0.49	13	0.90	60.24	0.46	13	0.97

The difference in rates between fed and feeding bivalves was less in the presence of chlorine, indicating a reduction in respiratory costs associated with food assimilation (Table 7.6).

Analysis of covariance was again used to identify homogeneity or the lack thereof among slopes correlating RR with body size (Table 7.7). For all activity levels in non-chlorinated experiments, *b*-values were significantly alike between temperatures with common slopes of 0.70 for starved and fed individuals and 0.73 for those feeding on algae. With chlorine the size-rate relationship remained unaltered in fed bivalves, but in those feeding on algae, the synergistic effect of chlorine and elevated temperature resulted in higher RR in large than in small individuals.

Subsequent NK tests showed that when starved (no chlorine), weight-specific rates were significantly dependent on temperature, increasing from 0.27 ml hr^{-1} g^{-1} at 15°C to 0.36 ml hr^{-1} g^{-1} at 25°C. This signifies no thermal acclimation during the 2-week starvation regime, as supported later by rate-temperature curves (Figs 7.2 & 7.3). Fed bivalves still showed temperature dependency between 15°C (0.37 ml hr^{-1} g^{-1}) and the two higher temperatures. Between 20 and 25°C rates were similar (0.45 & 0.46 ml hr^{-1} g^{-1} respectively) indicating some acclimation to high but sub-lethal temperatures in a non-chlorinated environment. When animals were feeding however, thermal independence of

Table 7.7. Effect of temperature with or without chlorine on OXYGEN CONSUMPTION RATES (RR, ml hr^{-1}) at three activity levels, when starved, fed or feeding: covariance analysis of the significant difference between slopes (*b*-values) and elevations (*a*-values) of allometric equations for RR at 15, 20 and 25 °C (see Table 7.6 for equations). Analysis procedure follows Zar (1982) using $\log_{10}$ transformed data.

ANALYSIS OF COVARIANCE $P < 0.01$

CONDITION	NO CHLORINE Between 15, 20 & 25°C					WITH CHLORINE Between 15, 20 & 25°C				
STARVED	k	DF	F_s	F	b_c	k	DF	F_s	F	b_c
b-values	3	126	0.27	4.78	0.70					
a-values	3	35	14.53	5.25						
FED										
b-values	3	126	0.37	4.78	0.70	3	126	2.79	4.78	0.69
a-values	3	128	25.35	4.78		3	128	87.31	4.78	
FEEDING										
b-values	3	126	1.18	4.78	0.73	3	126	8.90	4.78	
a-values	3	128	38.57	4.78						

NEWMAN-KEULS MULTIPLE RANGE TEST
(significant difference between a-values)

Between pairs (°C)	NO CHLORINE			WITH CHLORINE		
STARVED	q	p	$q_{0.01,126}$	q	p	$q_{0.01,126}$
15 & 20	6.69	2	3.70			
15 & 25	13.18	3	4.20			
20 & 25	6.48	2	3.70			
FED						
15 & 20	7.88	2	3.70	5.41	2	3.70
15 & 25	9.45	3	4.20	18.21	3	4.20
20 & 25	1.57	2	3.70	12.79	2	3.70
FEEDING						
15 & 20	0.02	2	3.70			
15 & 25	10.75	2	3.70			
20 & 25	10.77	3	4.20			

O_2 uptake shifted to between 15 & 20°C (0.45 & 0.46 ml hr^{-1} g^{-1} respectively), RR at 25°C being significantly higher.

In contrast to partial thermal acclimation shown by fed animals not exposed to chlorine, NK tests revealed that in those exposed, weight-specific O_2 uptakes were significantly temperature-dependent (Table 7.7). In feeding individuals, however, it was not possible to ascertain the significance of the temperature-related increases in rates, due to the lack of slope homogeneity. Nevertheless, the extent of the difference suggests no acclimation in these animals either.

In Table 7.8 the Student-*t* procedure tested for significant effects of chlorine alone by comparing regressional elevations and slopes at 15, 20 or 25°C. The decline in RR, which was significantly related to chlorine, occurred to the same extent in all sizes of fed *D. serra*. At 15 and 20°C, RR with chlorine was approximately 60 to 65% of that in the absence of chlorine. At 25°C, the reduction was less pronounced, the corresponding proportion being 82%.

Among feeding individuals at 15°C, *b*-values proved dissimilar so that the chlorine-associated decline in RR was more pronounced in large relative to small animals (Table 7.8). At 20 and 25°C, the size-rate correlation was not influenced by chlorine.

It thus appears that in the fed or feeding condition, the respiration rate in all sizes of *D. serra*, and at any of the three temperatures, showed no significant acclimation to the presence of chlorine even after two weeks exposure.

Table 7.8. Effect of chlorine at 15, 20 and 25°C on OXYGEN CONSUMPTION RATES (RR, ml hr^{-1}) when fed and feeding at a concentration of 20 - 25 X 10^6 *T. suecica* cells l^{-1}: Student-*t* analysis of significant difference between slopes (*b*-values) and between elevations (*a*-values) in allometric equations for RR at the same temperature with or without chlorine. Analysis procedure follows Zar (1982) using log10 transformed data.

STUDENT-*t* TEST
Comparing RR at the same temperature with and without chlorine

Temperature (°C) (*a*) Condition	Between slopes (*b*)				Betw elevations		
FED	t_s	$t_{0.01(2)}$	DF	b_c	t_s	$t_{0.01(2)}$	DF
15	1.37	±2.64	84	0.67	11.97	±2.64	85
20	-0.95	±2.64	84	0.71	14.99	±2.64	85
25	0.48	±2.64	84	0.71	6.39	±2.64	85
FEEDING							
15	4.22	±2.64	84				
20	-2.42	±2.64	84	0.75	9.69	±2.64	85
25	-1.26	±2.64	84	0.74	12.59	±2.64	85

Since CR displayed thermal acclimation whereas RR at the time of filter-feeding acclimated only partially, it would be an interesting exercise to contrast the volume of water cleared of algae against the concomitant volume of O_2 used (i.e. metabolic efficiency of filtration, V_w/V_{O2}). Strictly speaking this ratio has most significance when pumping rate is measured simultaneously with oxygen consumption (see Bayne, 1976). Nevertheless, some indication of V_w/V_{O2} is still attainable using the available data for CR (Table 7.1) and RR for feeding animals (Table 7.6). In Fig. 7.1 these data (for a 1-g animal) are plotted against acclimation temperature together with corresponding V_w/V_{O2} (which is assumed to be equivalent to CR/RR).

With respect to elevated temperature (no chlorine), CR/RR was relatively constant between 15 and 20°C, declining slightly from 2.4 at 20°C to 2.0 at 25°C. This decline resulted from a more rapid rise in RR than in CR.

Chlorine exposure drastically altered these efficiencies. Firstly, since RR and especially CR, were depressed by chlorine, all efficiencies were much reduced (<1.5). Furthermore, the relation with elevated temperature was reversed so that a temperature increase improved efficiency (0.3 at 15°C to 1.4 at 25°C). This reversal resulted from CR displaying a much sharper increase with temperature elevation relative to the concomitant rise in RR. It will also be noted that the magnitude of the

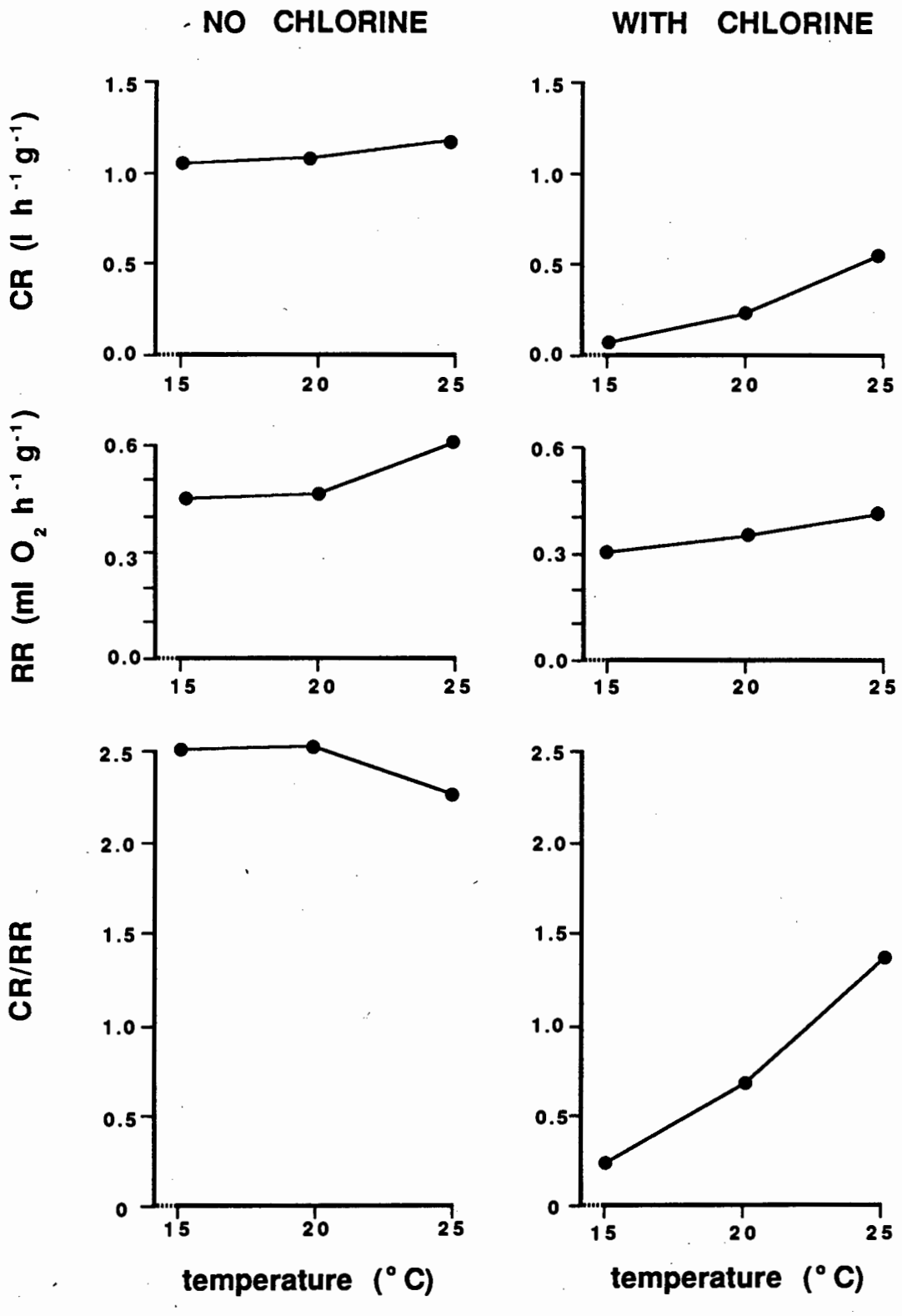

Fig. 7.1. Clearance and respiration rates (CR & RR) per gram dry tissue weight of an adult animal feeding on algae and the resultant metabolic efficiency of filtration (CR/RR) at the acclimation temperatures of 15, 20 & 25 °C without and with chlorine (0.1 - 0.3 ppm).

temperature-related change in efficiencies was much greater in the presence of chlorine.

Ammonia excretion

Ammonia-N excretion rates (U), monitored in fed animals, increased with temperature elevation regardless of the presence of chlorine, but at any one temperature, there was a chlorine-related suppression of U (Table 7.6). Weight-specific rates increased from 39 ug NH_4-N hr^{-1} g^{-1} at 15°C to 66 ug hr^{-1} g^{-1} at 25°C and on exposure to chlorine, the corresponding rise was from 5 to 60 ug hr^{-1} g^{-1}. The *b*-values in all allometric equations (0.45 - 0.55) were lower than in RR-regressions (0.65 - 0.78), indicating that U was less dependent on body size.

The low and variable ($r = 0.71$) ammonia excretion rates at 15°C in the presence of chlorine (Table 7.6) may be a consequence, in part at least, of the burrowing response of animals. On addition of chlorine, individuals burrowed deeply into the sand (20 cm depth) so that the exhalent siphon was not exposed on the surface as occurred in other experiments. Ammonia was therefore excreted directly into interstitial rather than overlying sea water. Since only the water above the sand was sampled, U in these animals may have been underestimated.

ANOCOVA and Student-*t* test demonstrated that neither an increase in temperature nor exposure to chlorine had a significant effect on the relationship between size and excretion rate (Table 7.9). NK tests showed that the

temperature dependency of weight-specific U was only significant between 15 & 25°C, whereas with chlorine added, a 5°C difference resulted in a significant increase.

Chlorine on its own significantly depressed U by 86% at 15°C with a smaller, but also significant, reduction of 24% at 20°C (Table 7.9, Student-*t*). At 25°C, however, there was only a slight non-significant chlorine-related reduction in U. It seems that at this temperature, the upper limit of thermal tolerance of *D. serra*, asymptotic excretion rates were reached that were independent of any further stress.

Changes in the rate of ammonia excretion are better understood when related to aerobic metabolism of fed *D. serra* by means of O:N ratios. Table 7.10 shows that under all test conditions, such ratios were positively correlated with body size, which is to be expected since *b*-values for ammonia excretion were always less than corresponding ones for respiration (see Table 7.6). Resultant weight coefficients were, nevertheless, low (<0.28) indicating that the difference in ratios between large and small animals was not substantial.

O:N ratios decreased with an increase in temperature, with or without chlorine, and at any one temperature (except 15°C) there was a chlorine-related decline in ratios. For example, in a 1 g animal not exposed to chlorine, ratios fell from 12 at 15°C to 11 and 9 at 20 and 25°C respectively. Ratios within this range indicate a greater proportion of protein catabolism relative to carbohydrate

Table 7.9. Effect of temperature and chlorine on AMMONIA EXCRETION RATES (U, ug NH_4-N hr^{-1}) of recently-fed *D. serra*: analysis of the significant difference between slopes (b-values) and elevations (a-values) of allometric equations for U at 15, 20 and 25°C with or without chlorine (see Table 7.6 for equations). Analysis procedure follows Zar (1982) using $\log_{10}$ transformed data.

ANALYSIS OF COVARIANCE $P < 0.01$

	NO CHLORINE Between 15, 20 & 25°C					WITH CHLORINE Between 15, 20 & 25°C				
Between	k	DF	F_s	F	b_c	k	DF	F_s	F	b_c
b-values	3	33	0.25	5.29	0.51	3	33	0.04	5.29	0.46
a-values	3	35	14.53	5.25		3	35	178.91	5.25	

NEWMAN-KEULS MULTIPLE RANGE TEST
(significant difference between a-values)

Between pairs (°C)	NO CHLORINE q	p	$q_{0.01,33}$	WITH CHLORINE q	p	$q_{0.01,33}$
15 & 20	2.76	2	3.89	65.57	2	3.89
15 & 25	5.42	3	4.46	79.24	3	4.46
20 & 25	2.66	2	3.89	13.66	2	3.89
	Conclusion: 15=20, 20=25			All sign. different		

STUDENT-*t* TEST
Comparing U at the same temperature with and without chlorine

Temperature (°C)	Between slopes (*b*) t_s	$t_{0.01(2)}$	DF	b_c	Betw elevations (a) t_s	$t_{0.01(2)}$	DF
15	0.84	±2.82	22	0.51	21.81	±2.86	19
20	0.64	±2.82	22	0.48	5.57	±2.86	19
25	0.34	±2.82	22	0.47	1.55	±2.86	19

and lipid (Conover & Corner, 1968; Bayne & Newell, 1983). Thus temperature elevation effectively increased the amount of protein being catabolised, an observation supported by biochemical data in Chapter 3 (see Fig. 3.1).

It can be inferred from the chlorine-related reduction in O:N ratios at 20 and 25°C that chlorine in combination with high temperatures, further promoted protein catabolism. This seems anomalous to the marked shift toward carbohydrate utilisation suggested by ratios at 15°C (+ chlorine). However, it is highly unlikely that a 5°C difference would result in such contrasting metabolism. It is more probable that ammonia excretion rates were underestimated. In fact, biochemical data in Chapter 3 suggested substantial protein catabolism at 15°C with chlorine.

Table 7.10. O:N ratios in relation to body size of individuals exposed to 15, 20 & 25°C with or without chlorine. The ratios are derived from allometric equations for rates of ammonia excretion and respiration in *D. serra* fed algae prior to experiments (see Table 7.6).

BODY SIZE	NO CHLORINE			WITH CHLORINE		
Dry wt (g)	15°C	20°C	25°C	15°C	20°C	25°C
0.100	8.379	6.574	5.344	36.500	4.519	4.426
0.500	10.656	9.298	7.473	48.408	7.026	6.618
1.000	11.807	10.756	8.648	54.767	8.459	7.879
3.000	13.918	13.548	10.898	66.748	11.377	10.371
5.000	15.039	15.077	12.125	73.158	13.060	11.779
Allometric Equations:						
a-values:	11.816	10.736	8.652	54.904	8.452	7.877
b-values:	0.149	0.212	0.210	0.178	0.271	0.250

RATE-TEMPERATURE CURVES

Acute temperature response

Acute responses of rates of oxygen consumption to temperature following acclimation to 15°C are presented, together with relevant Q_{10} values, in Fig. 7.2 for the three activity levels. Between 10 and 20°C, which approximate ambient conditions, Q_{10} values for starved individuals did not exceed 1.32 and above 20°C, values were slightly higher at 1.45. However, even though these low temperature coefficients signify some degree of acute thermal adjustment, it has been shown (Table 7.7) that even after 2 weeks at temperatures >15°C, RR remains significantly temperature-dependent.

In contrast, routine metabolic rates (fed and feeding) were more dependent on temperature between 15 and 25°C (Q_{10} range = 1.58 to 2.95), but less so between 10 and 15°C (Q_{10} values <1.3). The sharp temperature-dependency of routine rates is unlikely to be crucial to *D. serra*, as casual laboratory observations indicated short-term compensatory increase in feeding rate. Indeed, the degree of separation at any one temperature between O_2 uptake rate when starved and fed or feeding, would represent an estimate of activities such as those of filtration and assimilation of algal cells (Newell, 1979). This "scope for activity" was maximal at 25°C, a temperature above ambient but tolerated by *D. serra* for extensive periods (see Chapters 2 & 3).

Q_{10} VALUES	*Between temperatures (°C)*			
Activity level	10-15	15-20	20-25	25-30
Starved ——	1.12	1.32	1.45	1.14
Fed ·····	1.28	1.61	2.30	0.73
Feeding ——	1.17	2.95	1.58	0.94

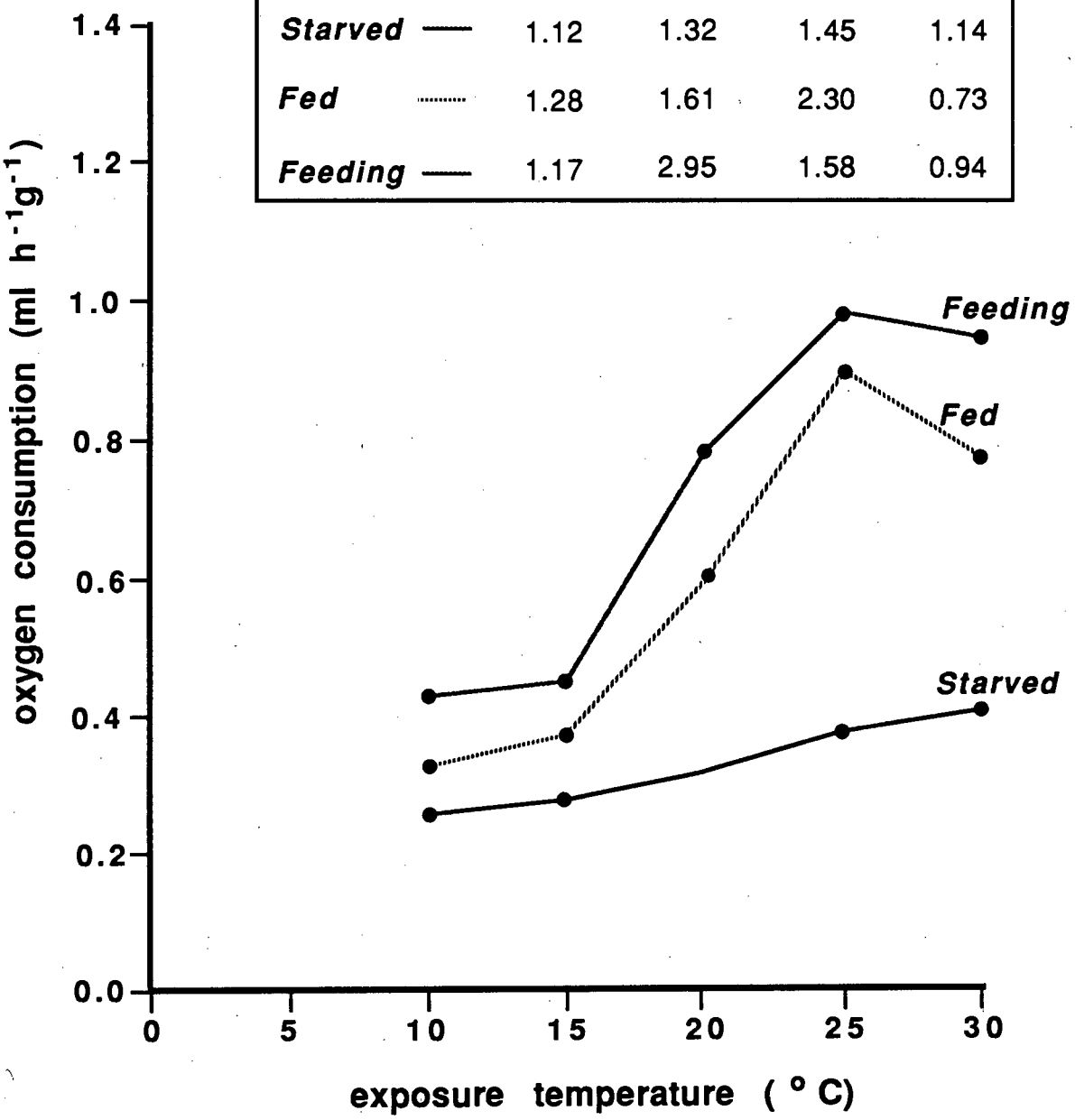

Fig. 7.2. Acute changes in respiration rate of adult *D. serra* acclimated to 15°C and then exposed to temperatures from 10 to 30°C. The three curves refer to rates per gram dry tissue weight when starved, fed and feeding on algae. Relevant Q_{10} values, calculated at 5°C intervals, are also presented.

Comparison between acute (Fig. 7.2) and long-term (Table 7.6) routine responses demonstrated the extent of compensatory adjustment following the 2-week acclimatisation period. For fed warm-acclimated animals, RR ranged between 0.37 and 0.46 ml hr^{-1} g^{-1} whereas acute RR increased from 0.37 to 0.91 ml hr^{-1} g^{-1} over the 15 to 25°C range. Corresponding rates when feeding on algae were 0.45 to 0.59 ml hr^{-1} g^{-1} for long-term exposure, compared to acute values of 0.45 to 0.98 ml hr^{-1} g^{-1}. By contrast, therefore, there is no doubt that *D. serra*, given sufficient time, could adapt its metabolic rate to an increase in environmental temperature. It should be borne in mind, however, that according to statistical interpretation, this would not amount to complete acclimation.

Nature of compensatory response

A series of acute rate-temperature curves are presented in Fig. 7.3 for fed individuals held at 10, 15, 20 & 25°C for 2 weeks before direct exposure to relatively lower or higher temperatures. It can be seen that acute RT curves were shifted to the right following long-term exposure to temperatures up to 25°C. Coincidence of this lateral translocation of RT curves with the increase in acclimation temperature demonstrated once again that acute respiration rates changed with time toward a new near-steady level (0.36 and 0.47 ml hr^{-1} g^{-1}) despite an increase in acclimation temperature from 10 to 25°C (note the acclimation temperature curve in Fig. 7.3).

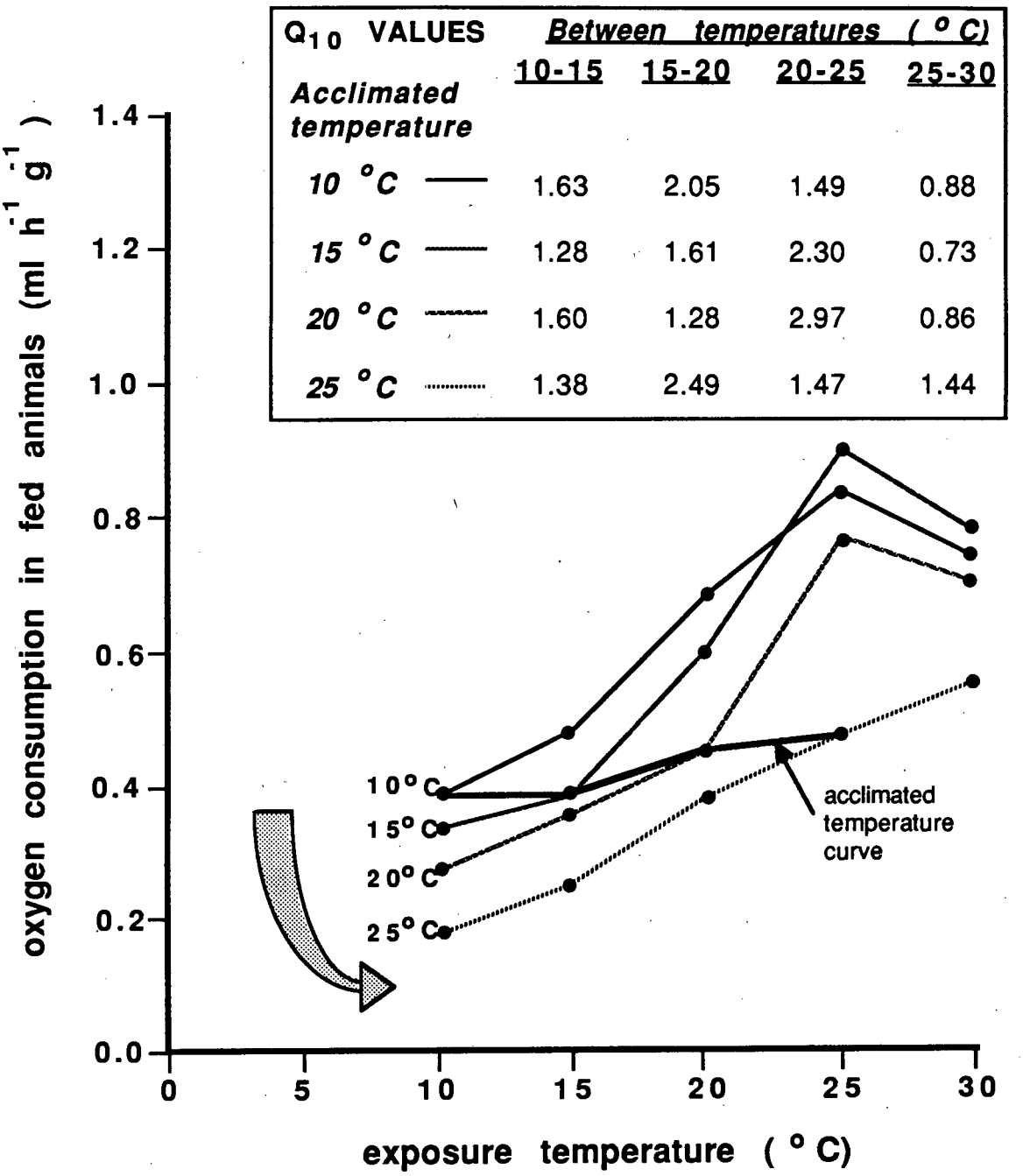

Fig. 7.3. Acute respiration rate-temperature curves for fed adult *D. serra* acclimated to 10, 15, 20 & 25°C for 2 weeks and then directly exposed to temperatures from 10 to 30°C. There is a resultant lateral translocation of the RT curves to the right (arrowed). The dark line joins data points at which acclimated equals exposure temperature. Relevant Q_{10} values, calculated at 5°C intervals, are also presented.

SCOPE FOR GROWTH AND REPRODUCTION

Interactive effects of body size, temperature and chlorine on the balance between energy acquisition and expenditure on a diet of *T. suecica* (20 - 25 X 10^6 cells l^{-1}) are presented in Table 7.11. The budgets, expressed in J hr^{-1}, identify rates of energy ingestion and absorption, energy loss in the form of respiration (for feeding animals), defaecation and ammonia-N excretion and finally, the subsequent gain or loss of energy for growth and reproduction (SFG). Allometric equations for these various energy-budget components were calculated from values in Table 7.11 and are presented in Table 7.12. Proportional amounts of energy used in each physiological process are shown in Fig. 7.4.

Although temperature increase did not result in an energy deficit, there was a decline in the amount of energy available for growth and reproduction in all sizes of *D. serra*. At 15°C SFG was 51 J hr^{-1} g^{-1} but at 20°C, this approximated only 22 J hr^{-1} g^{-1} and at 25°C, 10 J hr^{-1} (Table 7.12). The main factor contributing to such a decline was not the increase in metabolic rate, but rather the marked rise in energy loss in faeces or, reciprocally, the decline in AE (Fig. 7.4). The small temperature-related increase in ingestion rate did not compensate for this loss.

At any one temperature, the presence of chlorine drastically reduced SFG to marginally positive and/or negative values which no longer retained a clear linear relationship with body size (Table 7.11). All animals

Table 7.11. ENERGY BALANCE: Effect of temperature and chlorine on energy ingested and its use in the different physiological processes in relation to body size. The following conversion factors were used to obtain energy values: 17.336 J mg dry tissue weight $^{-1}$; 20.700 J mg dry algal weight $^{-1}$; 20.080 J ml O_2^{-1} (Gnaiger, 1983); 24.870 J mg NH_4-N $^{-1}$ (Elliot & Davison, 1975). The allometric equation for respiration rate while feeding at an algal ration of 5 - 6 mg DW l^{-1} was used here (see Table 7.6).

TEMPERATURE	BODY SIZE			ENERGY INGESTED		ENERGY ABSORBED	METABOLISM			ENERGY FOR GROWTH & REPRODUCTION
	Dry tissue weight (g)	Shell width (mm)	Tissue energy (kJ)	Dry weight (mg h^{-1})	Energy content (J h^{-1})	(J h^{-1})	Energy lost in faeces (J h^{-1})	Energy used in respiration (J h^{-1})	Energy lost in excretion (J h^{-1})	(J h^{-1})
15 °C NO CHLORINE	0.10	14.04	1.74	1.225	25.358	16.483	8.875	1.584	0.277	14.622
	0.50	24.19	8.67	3.066	63.466	41.253	22.213	5.383	0.667	35.203
	1.00	30.57	17.34	4.551	94.206	61.234	32.972	9.116	0.973	51.145
	3.00	44.32	52.01	8.513	176.220	114.543	61.677	21.010	1.773	91.760
	5.00	52.67	86.68	11.389	235.752	153.239	82.513	30.977	2.344	119.918
20 °C NO CHLORINE	0.10	14.04	1.74	0.807	16.705	5.880	10.825	1.797	0.421	3.662
	0.50	24.19	8.67	2.699	55.869	19.666	36.203	5.634	0.925	13.107
	1.00	30.57	17.34	4.539	93.957	33.073	60.884	9.217	1.299	22.557
	3.00	44.32	52.01	10.347	214.180	75.392	138.788	20.106	2.227	53.059
	5.00	52.67	86.68	15.177	314.164	110.586	203.578	28.896	2.860	78.830
25 °C NO CHLORINE	0.10	14.04	1.74	1.012	20.951	4.586	16.363	2.202	0.535	1.851
	0.50	24.19	8.67	3.173	65.689	14.386	51.303	7.131	1.177	6.078
	1.00	30.57	17.34	5.191	107.454	23.532	83.922	11.827	1.652	10.053
	3.00	44.32	52.01	11.324	234.410	51.336	183.074	26.374	2.831	22.131
	5.00	52.67	86.68	16.275	336.891	73.779	263.112	38.294	3.636	31.849
15 °C + CHLORINE	0.10	14.04	1.74	0.077	1.594	1.148	0.446	1.296	0.046	-0.194
	0.50	24.19	8.67	0.233	4.823	3.473	1.350	3.749	0.098	-0.374
	1.00	30.57	17.34	0.375	7.763	5.589	2.174	5.924	0.136	-0.471
	3.00	44.32	52.01	0.797	16.498	11.879	4.619	12.231	0.228	-0.580
	5.00	52.67	86.68	1.131	23.412	16.857	6.555	17.136	0.289	-0.568
20 °C + CHLORINE	0.10	14.04	1.74	0.364	7.540	4.147	3.393	1.083	0.344	2.721
	0.50	24.19	8.67	0.776	16.066	8.836	7.230	3.825	0.721	4.290
	1.00	30.57	17.34	1.075	22.253	12.239	10.014	6.586	0.992	4.661
	3.00	44.32	52.01	1.802	37.293	20.511	16.782	15.585	1.643	3.283
	5.00	52.67	86.68	2.290	47.413	26.077	21.336	23.261	2.079	0.737
25 °C + CHLORINE	0.10	14.04	1.74	0.869	17.814	4.809	13.005	1.339	0.519	2.951
	0.50	24.19	8.67	1.784	36.954	9.978	26.976	4.553	1.089	4.336
	1.00	30.57	17.34	2.442	50.584	13.658	36.926	7.711	1.498	4.449
	3.00	44.32	52.01	4.017	83.209	22.466	60.743	17.771	2.483	2.212
	5.00	52.87	86.68	5.063	104.676	28.317	76.559	26.201	3.141	-1.025

Table 7.12. a - and b - values calculated for different physiological processes (J h^{-1}) in relation to body size (g DW) at 15, 20 & 25 °C with or without chlorine (0.1 - 0.3 ppm) . Regressions take the form of Physiological rate = a (Body size)^{b} and have been calculated from values presented in Table 7.11. a = intercept; b = slope.

PHYSIOLOGICAL PROCESS	NO CHLORINE						WITH CHLORINE					
	15 °C		20°C		25°C		15 °C		20°C		25°C	
	a	b	a	b	a	b	a	b	a	b	a	b
Energy ingested	94.21	0.57	93.95	0.75	107.45	0.71	7.76	0.69	22.25	0.47	55.58	0.45
Energy absorbed	61.24	0.57	33.07	0.75	23.53	0.71	5.59	0.69	12.24	0.47	13.66	0.45
Energy lost in faeces	32.97	0.57	60.88	0.75	83.92	0.71	2.17	0.69	10.01	0.47	36.92	0.45
Energy used in respiration	9.12	0.76	9.22	0.71	11.83	0.73	5.92	0.66	6.59	0.78	7.71	0.76
Energy lost in excretion	0.97	0.55	1.30	0.49	1.65	0.49	0.14	0.47	0.99	0.46	1.50	0.46
Energy for growth & reproduction (SFG)	50.80	0.54	22.43	0.78	9.97	0.73	------	------	------	------	------	------

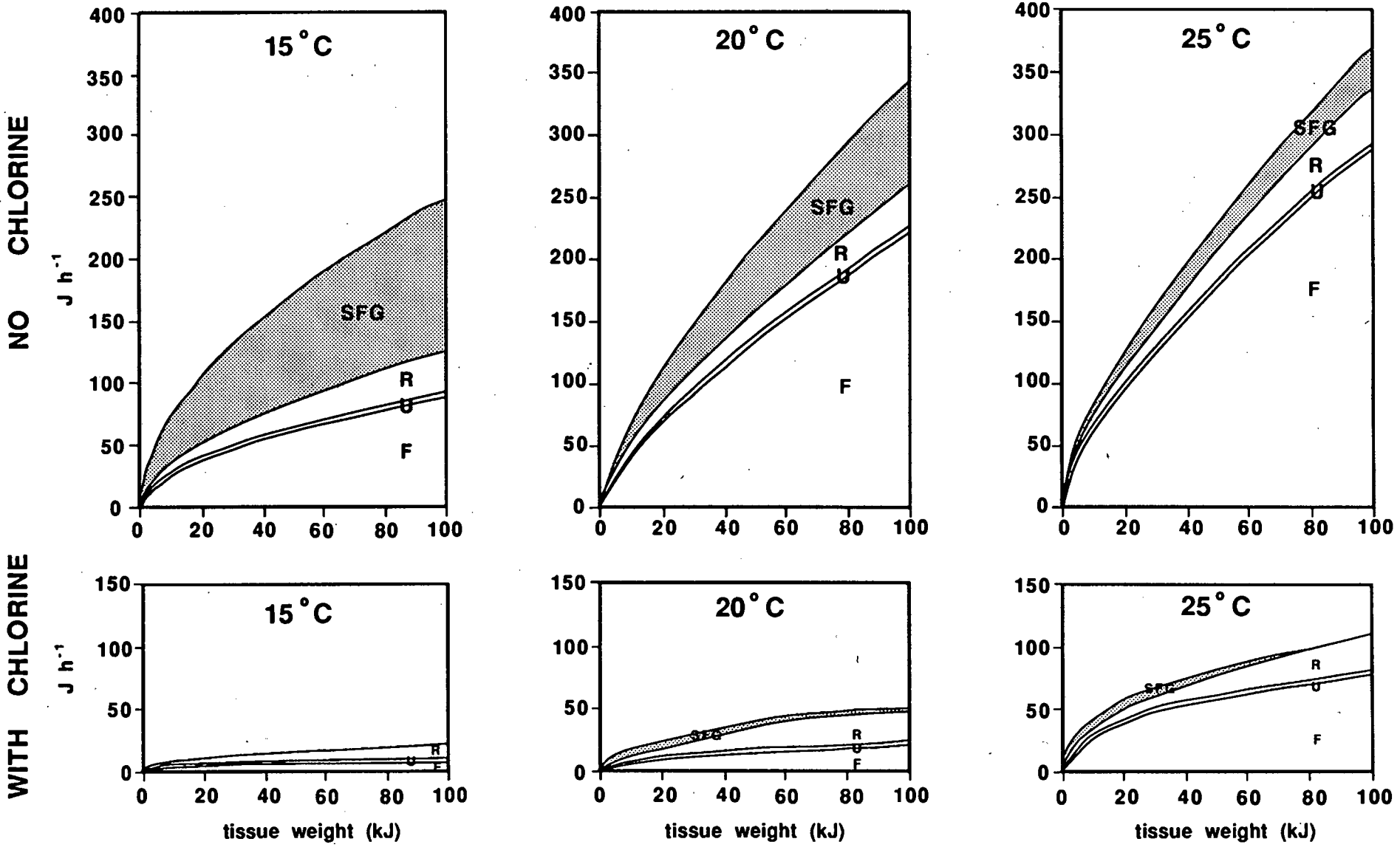

Fig. 7.4. Effect of temperature and chlorine (0.1 - 0.3 ppm) on size-related proportional utilisation of ingested energy in defaecation (F), excretion (U) and respiration (R). The shaded areas represent energy available for growth and reproduction (SFG).

displayed negative energy balance at 15°C, the deficit being more apparent in the larger individuals. At 20 and 25°C, SFG was mostly positive, with *Donax* of 1 g maintaining the highest energy credit.

Negative SFG at 15°C was chiefly due to the marked chlorine-related reduction in clearance rates and hence ingestion. The slight energy credit at 20 and 25°C, despite the presence of chlorine, arose from the temperature-related increase in ingestion which proved sufficient to offset losses via respiration, egestion and excretion (Tables 7.11 & 7.12; Fig. 7.4).

Energy loss associated with ammonia excretion was relatively negligible at all temperatures with or without chlorine (Table 7.11. Fig. 7.4). Underestimation of U at 15°C in the presence of chlorine would not, therefore, significantly alter estimates of SFG nor detract from conclusions reached on the effects of temperature and chlorine on the physiological fitness of *D. serra*.

GROWTH EFFICIENCIES

The association between gross (K_1) plus net (K_2) growth efficiencies and body size in relation to temperature elevation with or without chlorine is shown in Fig. 7.5.

At 15°C both K_1 and K_2 were a decreasing function of body size. However, with a rise in temperature to 20 and 25°C, larger rather than smaller *D. serra* displayed the greater relative growth efficiencies. Gross efficiencies

tended to decline by approximately 30 to 40% with elevation from 15 to 20°C. Exposure to 25°C, however, resulted in K_1 values being 20% higher than at 20°C. This result reflects accelerated ingestion of algae at 25°C, but ignores the simultaneous sharp decline in absorption efficiencies.

Absorption efficiencies are, of course, considered in the calculation of net growth efficiencies. K_2 values were thus higher and showed a negative correlation with temperature elevation. Size-related efficiencies ranged from 78 to 90% at 15°C, between 60 and 70% at 20°C and 40 to 44% at 25°C. These ranges also demonstrate that temperature elevation weakened the size-dependency of K_2 efficiencies.

Growth efficiencies changed sharply on exposure to chlorine in combination with temperature elevation (Fig. 7.5). At 15°C, K_1 and K_2 were negative for all sizes, the smaller individuals showing the greater adverse effect. At 20 and 25°C, efficiencies tended to be positive except in large *D. serra* (> about 4 g) at the higher of the two temperatures. Temperature-related increases in ingestion sufficient to offset simultaneous increases in energy losses, allowed *D. serra* to attain these positive growth efficiencies. Nonetheless, at any one temperature, gross and net efficiencies were lowered by exposure to chlorine.

Both K_1 and K_2 values were far more dependent of animal size when exposed to chlorine. Furthermore, this dependency tended to be a decreasing rather than an increasing function of body size at 20 and 25°C. Efficiencies were slightly

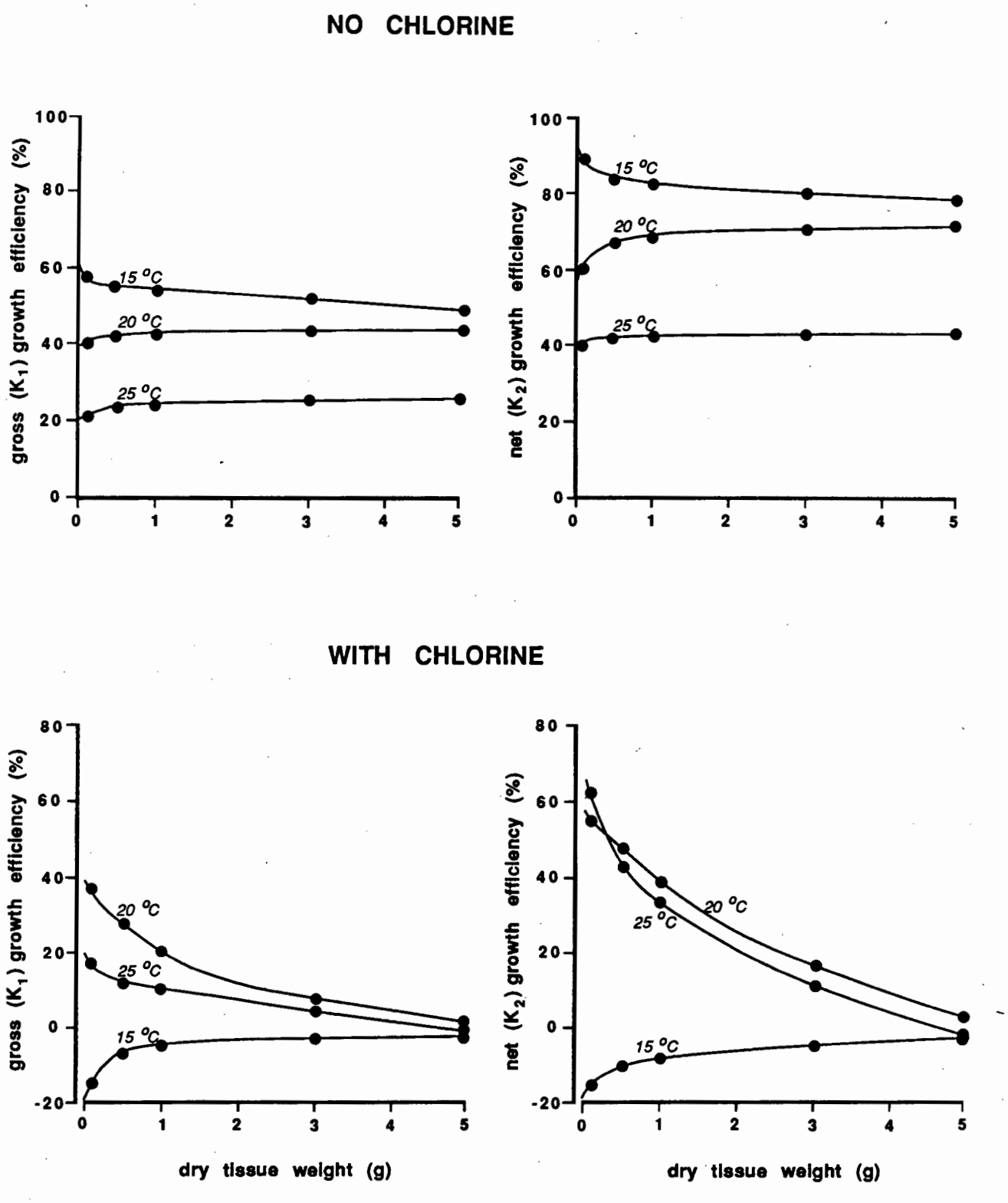

Fig. 7.5. Growth efficiencies as gross (K_1) and net (K_2) percentages in relation to body size (g DW) at 15, 20 & 25°C with or without chlorine. Gross = SFG/IR and net = SFG/AR based on data in Table 7.11.

higher at 20°C than 25°C as a consequence of greater absorption efficiencies and lower energy losses.

In more general terms it becomes apparent that with chlorine present, optimum SFG and growth efficiencies were attained at 20°C but without chlorine, optima occurred at 15°C. It should be borne in mind, however, that the magnitude of these optima differed enormously.

DISCUSSION

FOOD ASSIMILATION

This study has shown that temperature increase from 15 to 25°C resulted in an insignificant ($P<0.01$) increase in clearance and ingestion rates in *D. serra* of all sizes acclimated to experimental conditions for 2 weeks. This acclimated response was, however, subject to maintaining the ration quantity and quality at the optimum for maximum clearance rates at 15°C. Such ration control was necessary since CR is strongly influenced by the nature of diet (Chapter 4) and this sensitivity may well have obscured effects of temperature as well as chlorine.

It has generally been found among filter-feeding molluscs that clearance rates increase with acclimation temperature up to some optimum thermal level beyond which there is a relatively rapid decline (Newell & Kofoed, 1977b; Newell *et al.*, 1977; Buxton *et al.*, 1981; also reviews by

Newell & Branch, 1980; Bayne & Newell, 1983; Griffiths & Griffiths, 1987). In these studies the extent of thermal adjustment in CR of a particular species was dependent on the natural temperature range of that species.

The only other bivalves that have shown temperature-independent CR like *D. serra*, have been *Mytilus edulis* (between 10 and 20°C) [Theede, 1963; Widdows & Bayne, 1971; Widdows, 1973b; Thompson & Newell, 1985] and *Choromytilus meridionalis* (12 to 25°C) [Griffiths, 1980a]. In *M. edulis*, respiration rate also acclimated, so in effect this animal was still able to maintain a near-stable energy balance at elevated temperatures. *C. meridionalis* was more like *D. serra* in that RR did not acclimate and consequently thermal stability in CR did not have the same energetic advantage in these animals as it did for the mytilid species.

While CR in *D. serra* displayed thermal acclimation, the other measured component of energy gain, absorption efficiency, was drastically suppressed, falling from 65% at 15°C to 22% at 25°C and thus resulting in a highly significant decline in the proportion of food absorbed. Published data on the nature of temperature-AE associations are inconsistent, either indicating an increase (Winter, 1969, 1977; Elvin & Gonor, 1979), decrease (Widdows & Bayne, 1971) or steady AE (Buxton *et al.*, 1981) with temperature elevation. To some extent this discrepancy may be associated with the fact that absorption efficiency is functionally interrelated with many variables; gut capacity,

gut passage time, digestive efficiency and ingestion rate (Bayne & Newell, 1983; Hawkins & Bayne, 1984; Bayne *et al.*, 1984, 1987). The relative effects of increased temperature on any of these variables may differ between species (Griffiths & Griffiths, 1987).

It is nevertheless apparent that the pre-ingestive activity of *D. serra*, involving the gill filaments and labial palps, was not adversely affected by high temperatures, whereas post-ingestive processes were disrupted. The main functional components of post-ingestive activity would be gut capacity and the residence time of food in the gut (refer to discussion in Chapter 4).

In *D. serra*, shorter gut-residence times were associated with high temperature. This would lead to rapid processing of food in the intestine and especially in the digestive gland. Evidence of a decline in the duration and possibly the periodicity of the digestive gland cycle, was apparent in the increased amount of undigested material egested by warm-acclimated animals. The duration and periodicity of digestion forms part of a phasic rhythm that has been well-documented in bivalves, but usually only in association with the quantity and quality of food rather than with temperature (Morton, 1973; Mathers, 1974; Hawkins & Bayne, 1984; Bayne *et al.*, 1984, 1987).

The apparent association between high temperature and digestive efficiency no doubt also involves changes in the functional activity of digestive enzymes. The little

research which has been undertaken in this field points towards enhancement of enzyme activity with increase in temperature rather than its suppression, as suggested by the decline in AE in *D. serra* (Seiderer & Newell, 1979; Newell *et al.*, 1980). Newell *et al.* (1980) found that a number of alpha-amylase isoenzymes were associated with the crystalline style of *C. meridionalis*, which displayed different thermal optima over the acclimated temperature range 8 to 22°C. It was found that alpha-amylase increased the amount of glucose released from the breakdown of protein. The authors suggested that this would enhance AE but this was not substantiated. Since tissue extracts of digestive gland, style and gut from *D. serra* have all shown intense alpha-amylase activity (Krohn, 1987), it would be of interest to investigate the association between AE, alpha-amylase activity and temperature.

Chlorine exposure, even in the sublethal range 0.1 to 0.3 ppm (Chapter 2), resulted in a decline in clearance and ingestion rates in *D. serra*. At the same time these reduced rates became strongly temperature-dependent in sharp contrast to the independence of chlorine-free feeding rates. The most dramatic reduction in CR of 93% was observed at ambient temperature (15°C) rather than at 20 and 25°C.

Clearance rates were very low at 15°C due to intermittent valve and siphon closure. These postural changes indicated attempts to physically isolate tissues from chlorine. Such tissue isolation seems common among

bivalves exposed to atypical chemical changes in the environment, whether these are chlorine (Block, 1977; Chapter 2), copper or hydrocarbons (Akberali & Black, 1980; Trueman & Akberali, 1981; Trueman, 1983; Widdows & Johnson, 1988) or low salinities (Akberali & Davenport, 1982; Navarro, 1988). In the bivalves *Choromytilus chorus* (Navarro, 1988) and *M. edulis* (Widdows & Johnson, 1988) similar postural changes were also associated with depressed clearance and ingestion rates.

The synergistic effect of a chemical pollutant and high temperature on CR and IR has not received attention in the literature. In the case of *D. serra*, it seems that high temperatures impeded attempts to physically isolate tissues because of the valve gape required to maintain a feeding and ventilating current to counteract temperature stress.

The temperature-related decline in absorption efficiencies remained unchanged in the presence of chlorine. However, the proportion of food absorbed, although always much less than in chlorine-free experiments, increased slightly with higher temperatures. This was simply because the decline in AE was less than the corresponding temperature-related increase in ingestion rates.

Few other studies have considered similar effects of a change in the chemical environment on the absorption efficiencies of bivalves. Navarro (1988), investigating a wide range of salinities with respect to *C. chorus* and Widdows & Johnson (1988), measuring the effects of copper

and hydrocarbons on *M. edulis*, also found that absorption efficiencies were unaffected by these environmental changes.

METABOLIC EXPENDITURE

Metabolic expenditure in *D. serra*, measured as rates of aerobic respiration (in starved, fed or feeding animals) and ammonia excretion rates (in fed individuals), increased with raised temperature. Some of these rate differences were, however, insignificant between 15, 20 and 25°C. It was assumed that this indicated partial acclimation following 2 weeks of exposure to experimental conditions.

The fact that acclimated O_2 uptake rates were notably lower than corresponding acute thermal responses at 20 and 25°C, supported the statistical evidence of limited metabolic compensation in fed and feeding *D. serra*. Starved individuals, on the other hand, did not acclimate and even though their metabolic expenditure was much lower than those of fed bivalves, this lack of compensation would increase demands on body reserves at high temperatures.

However, given the natural particulate concentrations in surf waters at Ouskip (Chapter 4), it is doubtful that *D. serra* would face starvation in its own habitat at any time of the year. This may account for the inherent inability among starved animals to show thermal acclimation. It is however, more common in the literature to find starved bivalve molluscs acclimating to elevated temperature, particularly in north-temperate species, which often

experience marked seasonal variability in temperature and food supply. Thermal conservation in this context would help conserve energy (see Newell, 1979; Bayne & Newell, 1983).

Evidence of complete thermal acclimation of routine rates among bivalve species is usually only evident over a restricted temperature range (associated with ambient conditions) beyond which compensatory mechanisms appear to break down. In *D. serra* partial compensation may indeed be evidence of such a breakdown in complete acclimation considering that experimental temperatures (selected to reflect conditions in the thermal plume) exceeded the ambient range of 8 - 17°C (Walker *et al.*, 1984). Evidence of complete acclimation within the ambient range was provided by the recording of identical routine respiratory rates at 10 and 15°C following 2 weeks exposure to these temperatures.

The main function of acclimation, whether complete or partial, is nonetheless to optimise the energy balance of an organism experiencing environmental change. In the case of *D. serra*, the ability to reduce metabolic expenditure at temperatures above normal would be an energetic advantage to those animals residing within the influence of the power station plume.

The introduction of chlorine disrupted thermal compensation in that routine rates became strongly temperature dependent, and at the same time markedly

reduced. Starved animals appeared most adversely affected since aerobic respiration rates were not measurable. Presumably under these conditions there was a switch to anaerobiosis, a physiological adaptation well developed in *D. serra* (see discussion in Chapter 5).

Clearance and respiration rates seemed more interdependent when chlorine was administered. Both rates were depressed and both became strongly temperature dependent. This contrasts to the relative uniformity of CR coupled with only partially acclimated RR in a chlorine-free environment. From the rapid temperature-induced rise in metabolic efficiency of filtration with chlorine, it was apparent that food intake (energy gain) more than compensated for increased metabolism (energy expenditure) This provided a means of stabilising energy balance between 15 - 25°C. Such parallel adjustments in CR and RR have often been recorded as a compensatory mechanism among bivalves, but usually in response to high temperatures alone rather than in combination with a contaminant (Newell *et al.*, 1977; Newell, 1980; Buxton *et al.*, 1981; Shumway & Koehn, 1982).

Temperature-related increases in ammonia excretion rates in *D. serra* demonstrated only partial acclimation, as indicated by the lack of a significant difference in rates from 15 - 20°C and 20 - 25°C (ANOCOVA, $P<0.01$). Even after 2 weeks, U at 25°C remained significantly higher than at the ambient temperature of 15°C and this lack of acclimation

over a 10°C increase, together with the decline in O:N ratios, indicated increased utilisation of protein as a respiratory substrate to meet increased metabolic demands. This contention is supported by data in Chapter 3 which demonstrated that protein is the main body reserve in *D. serra* and that it is utilised faster and to a greater extent than either carbohydrates or lipids at high temperatures.

Among bivalves there are examples of excretion rates either acclimating (Ansell & Sivadas, 1973), remaining temperature dependent (Bayne & Scullard, 1977a; Jordan & Valiela, 1982; Thompson & Newell, 1985) or, like *D. serra*, demonstrating partial acclimation (Mann & Glomb, 1978). These differences could be attributed to contrasts in ambient temperature regimes together with the interaction between endogenous features such as nutritional and reproductive condition and phasic activity of the digestive gland (refer discussion in Chapter 5). Given so many dependable variables, it is not surprising that published data are inconsistent. Whether or not a species displays compensatory adjustment of U, the most relevant criterion remains the optimisation of physiological fitness.

Addition of chlorine slowed down ammonia excretion but at the same time effected a decrease in O:N ratios at 20 and 25°C. This was indicative of further protein catabolism. Changes in gross biochemical composition (Chapter 3) confirmed that the added stress of chlorine enhanced the decline in protein reserves. Bayne & Thurberg (1988) found

similar changes in O:N ratios in *Nucula tenuis* and *Mytilus edulis* in response to contamination by hydrocarbons and copper.

SFG AND GROWTH EFFICIENCIES

Interactive regulation between clearance, respiration and excretion rates resulted in a positive energy balance in all sizes of *D. serra* over the range 15 to 25°C. The amount of energy in credit, however, decreased with temperature elevation, principally because of the extensive decline in AE. As a consequence of diminished SFG, growth efficiencies also declined, the effect being most severe in small *D. serra* of <1 g.

Given the magnitude of the decline in SFG (up to 5-fold) and growth efficiencies (between 20 - 40%), it becomes obvious that a ration higher than the optimum for SFG at 15°C (5 - 6 mg DW l^{-1}; see also Chapter 6) would be required before warm-acclimated animals could anticipate a similar energy gain. *D. serra* exposed to 25°C would need a ration amounting to 7.0 - 8.6 mg DW algae l^{-1} to fulfill a necessary 39% additional intake amounting to 35 J hr^{-1}. The corresponding ration at 20°C would be 6.5 - 8.0 mg l^{-1} to effect a 29% increase in ingestion, equivalent to 26 J hr^{-1}. It follows that with the required increase in optimum ration, that for basic maintenance would also increase with temperature.

These estimates naturally assume that in relation to an increase in algal concentration at 20 and 25°C, there was no meaningful change in the rates of energy acquisition and expenditure. This assumption probably holds for AE, RR and U, all of which were more dependent on a 5 to 10°C increase in temperature than on an increase in ration from 6 to 8.6 mg DW l^{-1} (Chapters 4 & 5). CR on the other hand, would undoubtedly decelerate somewhat since it is independent of temperature but strongly influenced by algal concentration; for example with a ration increase as proposed here, a 50% decline in clearance was recorded at 15°C (Chapter 4). Nevertheless, the dominant constraint on the extent to which an increase in ration may compensate for temperature-related increases in metabolic losses, would be incipient overloading of the filtration mechanism at around 8 - 9 mg algae l^{-1} (Chapter 4).

While it is of interest to speculate on the energetic advantage of higher ration levels during thermal stress, *D. serra* may not be able to make full use of this additional energy source for the purpose of optimising SFG. In a study on the synergistic effects of temperature and algal concentration in *M. edulis* it was found that SFG was also markedly depressed above ambient temperature and although an increase in ration did improve this balance, the optimal level evident at ambient temperature was never attained (Widdows, 1978b).

Within normal temperature ranges most bivalves appear to regulate energy gain and loss so that SFG is optimised (Widdows & Bayne, 1971; Bayne *et al.*, 1976; Newell *et al.*, 1977; Newell, 1980; Buxton *et al.*, 1981; Thompson & Newell, 1985). In these circumstances SFG remained strongly dependent on ration level but relatively independent of temperature, indicating that in the natural environment, food availability rather than thermal level, probably has the most profound influence on growth (Bayne & Newell, 1983). This is probably true of *D. serra* as well, given the highly significant relationship between SFG and ration level (Chapters 4 & 6) and the indication that energy gain and expenditure are probably independent of temperature within the ambient range. It is also likely that at temperatures above ambient, but not greater than 25°C, food availability may remain the primary influence over growth and reproduction. With respect to *D. serra* of 1 g DW, this becomes apparent on noting the scale of difference between the SFG range in relation to algal concentrations at 15°C (0.3 - 66.07 J hr^{-1} on 2.5 - 7.5 mg DW l^{-1}, Chapter 6) and SFG maintained between 15 and 25°C on an optimal ration (10 - 51 J hr^{-1} on 5 - 6 mg DW l^{-1}).

The physiological sensitivity of *D. serra* to chlorine was dramatically demonstrated by the decline in SFG to marginally negative and/or positive values. The principle reason for this impoverished energy balance was a much reduced, but positively temperature-correlated CR in

combination with the temperature-related decline in AE. Despite the decrease in AE, SFG increased slightly with temperature elevation, although this was dependent on animal size. Indeed, growth efficiencies were negative at 15°C but positive and highest (albeit still markedly depressed by chlorine) at 20°C.

SFG data were, to some extent, inconsistent with gross biochemical changes in body reserves (Chapter 3). At 15°C, loss of body tissue was predicted by both sets of data, but at 20 and 25°C, energy balance indicated slight growth, whereas biochemical composition demonstrated marked reserve depletion coupled with weight loss. This anomaly probably has its origin in the completely different methods used to establish body condition. SFG is a physiological integration whereas biochemical indices rely on direct measurements. Nonetheless, both sets of data showed that irrespective of temperature, chlorine impaired growth and reproduction.

SFG has proved a useful tool on the rare occasions it has been applied in pollution studies, since its impairment provides an excellent indicator of sub-lethal contamination (Bayne *et al.*, 1981; Widdows *et al.*, 1981; Widdows & Johnson, 1988). Certainly SFG in *D. serra* showed a susceptibility to sublethal chlorine levels (0.1 - 0.3 ppm) that was not evident in experiments on survival and burrowing responses and only partially indicated by heart beat frequency (Chapter 2). While gross biochemical change

also provided a clear signal of chlorine contamination, the tedious and time-consuming methodology makes it an unfavourable research technique.

ECOLOGICAL CONSEQUENCES

The physiological consequences of elevated temperature and chlorination on individual *D. serra* have been clearly demonstrated in the present study. It becomes pertinent at this point to consider the ecological significance of a reduction in scope for growth and reproduction with respect to the *Donax* population living in the path of the thermal plume from Koeberg Nuclear Power Station.

The impaired growth efficiencies originating from the decline in SFG under conditions of optimal food availability and high temperature, with or without chlorine, would inevitably lead to smaller sized *D. serra*. In view of the fact that *D. serra*, in common with other bivalves (Griffiths & Griffiths, 1987), increases gamete production (P_r) with an increase in body size (Chapter 6), suppressed growth would reduce the amount of reproductive material released by the sexually mature sector of the population.

For instance, a 3 g fully mature individual in its natural environment can be expected to have a total annual production value (P) of 59 kJ, of which 33 kJ would constitute reproductive material (P_r) [Table 6.7]. Assuming that P reflects an ambient net growth efficiency (K_2) around 85% (Fig. 7.5), it follows that at 20°C (K_2 = 70%) and 25°C

(K_2 =40%) P would approximate only 49 and 28 kJ yr^{-1} respectively. If reproductive effort (P_r/P) is not considered to alter with temperature elevation, P_r would be reduced to between 16 and 27 kJ yr^{-1}. In areas of the plume where chlorine has not dissipated and remains in the range 0.1 - 0.3 ppm, even lower growth efficiencies can be anticipated. Consequently, between 20 and 25°C production in the region of 8 - 14 kJ yr^{-1} can be predicted of which 5 - 8 kJ would be reproductive output.

A study was undertaken by Bayne & Worrall (1980) comparing P_g and P_r between two separate *M. edulis* populations, one receiving a richer food supply while the other was exposed to warmed waters from an electrical-generating station. It was found that in the thermally stressed population both growth and reproductive output were lower. In addition, reproductive effort (P_r/P) was reduced from 60% to 26%.

There is some indirect evidence that *D. serra* may be able to buffer its reproductive effort against environmental stress. Examination of gonad material during sustained exposure to high temperatures with or without chlorine (Chapter 3), showed that gametogenic condition was virtually unchanged after 10 to 15 days of enforced stress. Such buffering has been demonstrated in *Choromytilus meridionalis* in relation to aerial exposure (Griffiths, 1981a, b). It was found that although P declined 50% between continuously submerged populations and those tidally exposed, the

proportion of production diverted to gamete output was maintained.

While laboratory studies provided a convenient measure of the extent of physiological impairment induced by high temperature and chlorine under optimal feeding conditions and sustained exposure, it should be realised that the field situation represents a more dynamic environment. Natural SFG would most likely be sub-optimal most of the time, even in *D. serra* distant from the plume (Chapter 4). In addition, SFG within the plume would not be subjected to sustained stress as imposed in the laboratory, but rather to more temporal variation in high temperatures and chlorine concentrations (Chapter 1). Predicted ecological consequences should, therefore, not be regarded as absolute, but rather as suggestive of the physiological aspects of the *Donax* population most likely to be adversely affected by the conditions in the thermal plume.

CONCLUSIONS

1.) Post-ingestive processes were markedly impaired by temperature elevation beyond the ambient range, the sharp fall in absorption efficiency being suggestive of a reduction in the duration and periodicity of the digestive gland cycle. On the other hand, pre-ingestive and ingestive activities were essentially unaltered by high temperature when the optimal algal ration was available. Feeding rate therefore did not compensate for the temperature-related decline in the proportion of food absorbed.

2.) The magnitude of increase in metabolic expenditure (respiration and excretion rates) with temperature elevation was such that partial acclimation was indicated. Such limited energy conservation in warm-acclimated animals was suggestive of a breakdown in ideal compensation that would otherwise characterise the ambient thermal range. At high temperatures, O:N ratios alluded to increased utilisation of protein as a respiratory substrate; this was further evidence of an inability to acclimate completely.

3.) Temperature-related adjustments in energy acquisition and expenditure were inadequate to compensate for the simultaneous sharp decline in absorption efficiencies. Consequently, even with an optimal ration available, SFG and its derivative, growth efficiency, although always positive, declined with an increase in temperature.

4.) At ambient temperatures, and even up to 25°C, food availability, rather than thermal level, appeared to be the

principle determinant of growth and reproduction. It is likely therefore that at sub-optimal rations SFG would be negative on exposure to temperatures in the plume.

5.) All physiological rates, besides those concerned with post-ingestive food processing, were markedly impaired by a sub-lethal dose of chlorine. Feeding rate, as well as metabolic expenditure, were dramatically reduced, but at the same time both became positively temperature dependent. Energy gain and loss were adjusted in parallel so that with temperature elevation and the concomitant decline in absorption efficiencies, energy balance stabilised between marginally negative and positive values.

6.) At ambient temperature, chlorine had the most retarding effect on growth efficiencies. Warmer conditions under these circumstances actually resulted in a relative improvement in efficiencies, especially in small, immature *D. serra*.

7.) From laboratory estimates of the effect of high temperature and chlorine on SFG and growth efficiencies, some likely ecological consequences can be predicted for the *Donax* population within the plume:

a.) Growth rate and asymptotic body size would be reduced, especially in the population sector nearest the outfall mouth where the highest temperatures and chlorine levels can be expected (see Chapter 1).

b.) As a consequence of a reduction in body size, reproductive output would be less, although there is indirect

evidence that reproductive effort per individual would be maintained.

c.) Points a and b above imply that both the production and physiological fitness of the *Donax* population would decline along the plume gradient from the seaward side to the outfall mouth.

8.) Although laboratory experiments provided clear unilateral responses to sustained high temperature and chlorine, it is acknowledged that in the field situation the environment within the plume would be far more variable. The complex interactions between food availability and the ambient plus plume temperature regime would result in both a dilution and magnification of the absolute measures obtained in laboratory experiments.

CHAPTER EIGHT

SYNTHESIS

This final chapter presents a synthesis of the most important results arising from research into the physiology of *D.serra*. It also summarises information relevant to improving the knowledge of marine bivalve physiology. There are two parts to this chapter. The first relates to the natural physiology and energetics of *D. serra* and the second, to physiological responses to elevated temperature and chlorine levels characteristic of the discharge plume from Koeberg Nuclear Power Station.

PHYSIOLOGY AND ENERGETICS

The most important physiological features of *D. serra* were those relating to biochemical composition, filter-feeding dynamics, metabolic expenditure and estimates of SFG.

Biochemical composition

Protein was identified as the main energy reserve in *D. serra*, with the gonads, mantle, foot and adductor muscles indicated as storage sites. Protein is also the main energy store in the swimming pectinids (Ansell, 1974b; Taylor & Venn, 1979; Barber & Blake, 1983; Epp *et al.*, 1988). In more sedentary bivalves such as oysters and mytilids, however, glycogen is the most important reserve (Gabbott, 1983). These findings led to the suggestion in Chapter 3 that only in bivalves containing extensive locomotor muscle , is protein content sufficient to constitute the main metabolic energy store. There is strong evidence that the foot and adductor muscles of *D. serra* respire anaerobically

(Trueman & Brown, 1987). Since protein can be catabolised anaerobically, as well as aerobically (Holland, 1978), the storage of this substrate in these tissues is a distinct metabolic advantage.

Filter-feeding dynamics

Availability of suspended material to *D. serra* is influenced by short-term cycles of upwelling and downwelling so that the most concentrated suspensions in the surf zone occur in winter. The potential food resource in the surf zone is comprised mainly of phytoplankton-derived detritus, augmented by phytoplankton during episodic blooms.

Using seafoam detritus from the natural environment rather than cultured algae as a food source in the laboratory, enabled a more realistic evaluation of the feeding dynamics of *D. serra*. This was because detritus best reproduced the quality of natural food material. In most other studies, attempts to reproduce ambient food quality have involved adding natural silt or unnatural inert material to cultured algae. It is more meaningful to use naturally-derived material such as seafoam detritus. The more recent approach of maintaining bivalves in unfiltered sea water from natural habitats (Lucas *et al.*, 1987; Matthews *et al.*, 1989), is the best way of realising the true significance of food quality and quantity in filter-feeding dynamics.

D. serra displayed contrasting behaviour when presented with foods of differing qualities. When feeding on a high

quality food source (algae), siphons were fully extended and open, but on a lower quality diet (detritus), the length and aperture of siphons were greatly reduced. Postural changes in siphons may result from a chemosensory response to food quality via ciliated sensory receptors on the siphons and mantle edge.

Food quality affected the manner in which *D. serra* regulated ingestion and assimilation of food at high particulate densities. Detritus-fed animals reduced clearance rates together with a small release of pseudofaeces. Those fed algae reduced clearance and increased pseudofaeces production or increased clearance at the same time as releasing copious amounts of pseudofaeces and faeces containing an abundance of undigested material. The ability to enhance particle rejection by rapid egestion of faeces containing undigested food is a feature not previously recorded among bivalves.

Some bivalve species regulate the quality of the ingested ration by pre-selecting nutritious particles at the gills and labial palps and rejecting poorer quality material as pseudofaeces, even at very low ration levels (Kiorboe & Mohlenberg, 1981; Mohlenberg & Kiorboe, 1981; Newell & Jordan, 1983). Since *D. serra* only produced pseudofaeces in response to high particle densities, pre-ingestive selection does not appear to play a role in regulating ration quality. Control was mainly achieved by increasing ingestion over a wide range of food concentrations coincidental with maximum

absorption efficiencies. Post-ingestive sorting was indirectly indicated as a likely means of improving the assimilation of detrital material.

Even though a diet of mono-cultured algae is recognised as artificial, the fact that this food is so efficiently ingested and assimilated indicates an ability on the part of *D. serra* to respond to the periodicity, duration, density and food value of phytoplankton blooms. *D. serra* could thus be seen as behaving opportunistically in its natural environment by maximising particle ingestion at times of both a moderate and abundant supply of high quality food.

Metabolic expenditure

Standard metabolism, rather than feeding activity accounted for most of the energetic expenditure associated with aerobic respiration in *D. serra*. This is in contrast to the high feeding, but relatively low maintenance costs, recorded in other bivalve species. Such differences are not necessarily species-specific but could relate to the nutritional history of an animal, to intrinsic fluctuations in ventilation rates and to the point at which, and the duration over which, oxygen uptake is monitored once food is introduced.

Food quality has a significant effect on the relationship between the size of *D. serra* and respiration rate. Large animals respire more slowly than small ones when feeding on a poor relative to an enriched food source. Food quality has not previously been reported as having a

size-related effect on oxygen uptake rates. More commonly, such an intraspecific variation is attributed to food quantity, temperature, salinity and activity patterns not associated with quality of diet.

Weight-specific respiration rates while feeding on detritus were notably lower than during the ingestion of algae. Using cultured algae in experiments can thus lead to an overestimation of the metabolic costs of clearance and ingestion, as well as digestion and assimilation of the ingested ration.

O:N ratios calculated from the atomic equivalents of oxygen consumed to ammonia excreted reflect considerable protein catabolism especially among starved animals. This supports the conclusion reached from data on gross biochemical composition that *D. serra* relies predominantly on protein as an energy reserve.

Anaerobic respiration appears to be an important part of overall metabolism in *D. serra*. Anaerobiosis has been indicated by low oxygen tensions in the pedal sinus while burrowing plus by the virtual absence of mitochondria in the foot (Trueman and Brown, 1987). Supporting evidence of pedal muscles functioning anaerobically in this study, was the absence of an increase in oxygen consumption during burrowing. Oxyconformity and the occurrence of an oxygen debt during hypoxia are further indications (Van Wijk *et al*, 1989). Reaction to unfavourable conditions involves withdrawing totally into its shell and since no oxygen

uptake can be measured under these conditions, metabolism must be largely anaerobic.

Estimates of SFG

Dietary quality and quantity profoundly influence short-term estimates of SFG in *D. serra*. At comparable ration levels, SFG on an algal diet surpasses that when feeding on seafoam detritus. This effect was most dramatic at optimum SFG which was 50 times greater when algae was used as food. The principle reasons for this contrast are reduced ingestion rates, and to a lesser extent, lower absorption efficiencies in detritus-fed animals.

Other studies have not recorded such a marked contrast in SFG in relation to food quality. Indeed some workers have recorded more favourable SFG with a food source of algae and silt (Mohlenberg & Kiorboe, 1981; Kiorboe *et al.*, 1981; Bayne *et al.*, 1987) or one of naturally derived particulates (Griffiths & King, 1979a; Stuart, 1982). Nevertheless, short-term estimates of SFG and growth efficiencies in these studies did not reach the maxima recorded for *D. serra* on an algal diet. It would appear therefore that *D. serra* shows an enhanced ability to maximise SFG in the short term when food value and quantity are optimal such as during a phytoplankton bloom.

Comparison between natural production based on field data and SFG as estimated in the laboratory, highlighted some important drawbacks of using the latter to evaluate actual growth. Cohort analysis of field data produced a

Gompertz growth curve which demonstrated two features not apparent from laboratory-based SFG. Asymptotic size is reached after 5-6 years and the growth rate declines with age. Furthermore, field data enabled estimation of the partitioning of total production between somatic and reproductive growth in different sized *D. serra*.

Optimal SFG, growth, efficiencies and P:B ratios on an algal diet overestimated production in the natural environment, whereas a detrital food source resulted in an underestimation. On comparing these results with those from other studies, it is apparent that the validity of SFG as an index of natural production is a function of the combination of food quality and quantity.

PHYSIOLOGICAL RESPONSES TO TEMPERATURE AND CHLORINE

Survival, burrowing response and heart rate.

Experiments designed to establish the survival rate and burrowing response of *D. serra* to high temperatures revealed that in terms of median mortality estimates, small individuals have a higher upper thermal tolerance than large individuals. This relates to the fact that small *D. serra* reside at mid-tide, a microhabitat which undergoes greater environmental change than the subtidal where adults are found. All sizes of *D. serra*, however, were forced to surface at high temperatures which are not necessarily lethal but which are characteristic of the power-station thermal plume.

On exposure to chlorine, *D. serra* responds by withdrawing into its shell and remaining closed for up to 6 days, depending on the chlorine concentration. This is a common avoidance response displayed by bivalves in response to environmental contamination, although the duration of valve closure varies between species. It is postulated that chemoreceptors on the siphons and mantle edge of *D. serra*, and other bivalve species, play an important role in the rapid closure response. Valve closure can result in dislodgement with lethal consequences if stranded. However, at low chlorine levels, valve closure was of short duration and *D. serra* soon returned to burrowing.

Heart rate frequency showed no acclimation at elevated temperatures, a characteristic feature among marine bivalves. On exposure to chlorine, heart rate dropped dramatically as the valves closed. It seems that a fall in heart rate is in response to a concomitant decline in oxygen tensions in the mantle cavity related to valve closure. Heart beat frequency recovered at low chlorine levels once the valves opened. Sublethal effects are only apparent at concentrations above 0.6 ppm in combination with raised temperatures.

Biochemical composition

Body reserves, especially protein, were extensively utilised to offset metabolic stress induced by high temperatures and chlorine. The extent of reserve depletion was similar to that recorded in many other bivalve species in response to

natural stress factors such as seasonal changes in temperature and food availability. Regardless of the origin of stress, it is clear that bivalves generally resort to stored reserves to meet the compensatory increases in energy expenditure. Although the nature of the reserve most utilised may differ between species (eg. glycogen or protein), the end result is the same; weight loss and after prolonged stress, gonad recession, followed by death.

Feeding, metabolic expenditure and SFG.

Feeding rates in *D. serra.* acclimated to elevated temperatures but at the same time, absorption efficiencies declined dramatically. This led to the conclusion that pre-ingestive and ingestive activities involving the gill filaments and labial palps are not adversely affected by elevated temperatures. On the other hand post-ingestive processes are impaired in a manner suggestive of a reduction in the duration and periodicity of the digestive gland cycle.

It is more common among bivalve species to find feeding rates increasing with raised temperatures. However, whether acclimation or an increase is demonstrated, the extent of thermal adjustment in feeding rate of any particular species appears dependent on the natural temperature range of that species. Rates of metabolic expenditure (respiration and excretion) displayed partial temperature acclimation. This limited energy conservation was suggestive of a breakdown in ideal acclimation that would otherwise be a feature of *D.*

serra within its natural temperature range. The temperature- related adjustments in feeding and metabolic rates were inadequate however, to compensate for the coincidental decline in absorption efficiencies. Consequently, SFG and growth efficiencies declined with an increase in temperature.

A sub-lethal dose of chlorine markedly depressed feeding and metabolic rates, but not absorption efficiencies. The subsequent balance between energy gain and loss was marginally negative or positive, depending on animal size and temperature. These SFG estimates were made at optimal algal rations. It is likely, therefore, that at sub-optimal rations, the synergistic effect of temperature and chlorine will result in a large energy deficit. In this context, food availability in the vicinity of the outfall may determine the extent to which *D. serra* tolerates high temperature and chlorine.

These SFG estimates for *D. serra* showed a susceptibility to chlorine that was not evident in experiments on survival and burrowing responses and only partially indicated by heart beat frequency. While gross biochemical changes also provided a clear signal of chlorine contamination, the tedious methodology makes it a less-attractive technique compared to SFG estimates.

Ecological consequences at the outfall

The most serious effect that the discharge plume is likely to have on *D. serra* is dislodgement from the sand in the

immediate vicinity of the outfall. In this area the substratum is heavily scoured by the effluent flow and it is unlikely that *D. serra* can maintain its position here. Furthermore, since the temperature and chlorine levels of the discharge can result in *D. serra* surfacing or closing its valves, even animals distant from the outfall current could face dislodgement by waves once anchorage is lost. Subsequent stranding on the beach will result in death from desiccation or predation.

There can be no doubt that *D. serra* inhabiting areas in the immediate vicinity of the outfall would experience physiological stress. The most likely consequences would be a reduction in growth rate and the maximum attainable body size. With a reduction in body size, reproductive output would be less, although reproductive effort per individual. is likely to be maintained. Recruitment into these areas may also be diminished. Although tolerances of larvae and spat were not investigated in this thesis, it is generally accepted that these life forms are more susceptible to environmental stress factors than adults (Newell, 1980). Therefore, selection for individual *D. serra* which can survive within the influence of the discharge plume may occur primarily at the settlement phase

In areas 200 - 600 m away from the outfall, temperatures and chlorine levels of the discharged sea water are much lower (see Chapter 1). Bivalves in these areas are unlikely to experience physiological stress or physical

displacement from the sand. In terms of the physiological aspects investigated in this thesis, it can be concluded that the warmed and chlorinated sea water from Koeberg Nuclear Power Station has a detrimental effect on *D. serra* only in an area confined to the immediate vicinity of the discharge outlet.

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